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THE EFFECTS OF LOW POWER LASER THERAPY UPON NON-COLLAGENOUS AND COLLAGENOUS PROTEIN CONTENT DURING THE HEALING OF SKELETAL MUSCLE TISSUE FOLLOWING ACUTE BLUNT TRAUMA

BY

PAULO DINIZ DE CASTRO MUNIZ

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE

DEPARTMENT OF PHYSICAL THERAPY

Edmonton, Alberta

FALL, 1992

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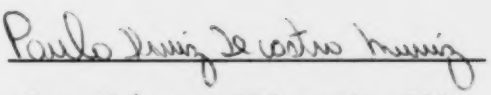

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Dr. David J. Magee

Dr. Brian D. Fisher

Dr. Thomas P. Martin

Dr. Sharon A. Warren

Date: April 28, 1992

DEDICATION

THIS THESIS IS DEDICATED TO MY WIFE DANIELLA AND

TO ALL THE ANIMALS USED IN SCIENTIFIC RESEARCH

ABSTRACT

Muscle injuries have increased with the number of participants and are responsible for athletes' absence from practice and games for various periods in which high cost rehabilitation is involved. Among muscle injuries, acute blunt trauma is frequent, but in practice, the extent of damage caused is difficult to measure scientifically. Low power laser therapy (LPLT) is being employed as a therapeutic modality by physical therapists. The purpose of this study was to determine the effects of LPLT (1 j/cm^2, daily x 10) upon acute blunt trauma to muscles in controlled experiments. A reproducible model of single acute blunt trauma to the medial gastrocnemius muscle of anaesthetized male rats (n=28) was used. The levels of non-collagenous protein content and collagenous protein content were determined 12 days post-trauma in control, injured, laser-control and laser-injured animals. There was no significant difference among all animals' muscle weight ($p > .05$) confirming that this model does not cause any alteration in the food intake and mobility of the animals which could influence the outcome measures of this study. The results demonstrated a general decrease in the levels of non-collagenous protein content, but this difference was not statistically significant ($p > .05$). A significant increase in the level of collagenous protein content ($p < .0005$) was observed. It was concluded that the LPLT protocol employed was not of maximum benefit during the time period outlined in this study (day 3 to day 12 post-

trauma) following acute blunt trauma. The data confirmed a trend for a decrease in the levels of non-collagenous and an increase in the levels of collagenous protein content following injury.

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CHAPTER ONE

INTRODUCTION

There has been a great increase in the number of sports participants at all levels of every sport and with it, the number of sports injuries has also increased. There is a high incidence of muscle injuries among athletes and these injuries constitute one of the greatest problems athletes face in their careers. Smodalaka (1979) has reported that ten per cent of all injuries treated at hospitals are sports injuries. Muscle injuries are responsible for athletes' absence from practices and games for varied periods of time in which high cost rehabilitation is involved (Prentice 1990). The extent of the muscle damage caused by a single impact trauma may be related to the amount of force delivered to the area. However, in practice, upon muscle injury, the amount of muscle damage caused is hard to determine or measure scientifically.

Physical therapists treat many muscle injuries that originate from sports activities or accidents. Acute blunt muscle trauma is frequent among these injuries. In order to have an understanding and to develop a rational for treatment, it is necessary to obtain information regarding the cellular events that are involved in the process of muscle healing. This information may be obtained from clinical and experimental studies. However, only a few experimental studies have been reported concerning these important cellular

events which occur during healing (Fisher et al. 1990).

Different therapeutic modalities such as the ultrasound, cryotherapy and recently and to a lesser extent, low powered laser therapy have been employed in the treatment of muscle injuries. However, the effects of these therapies upon muscle healing are not completely known and need to be studied in detail at the cellular level (Fisher et al. 1990).

Considering the characteristics of the muscles involved in an injury, one has to consider that muscles are composed of bundles of muscle fibres. These fibres in turn, are composed of contractile proteins which give the muscle the ability to shorten. Following muscle injury, there is a catabolic phase in which there is an enhanced protein degradation (Tishler and Fagan 1983, Fisher et al. 1990) and rapid proliferation of fibroblasts in the wounded area. A relatively small number of muscle fibres regenerate, but because of time and number of cells, there is a greater tendency for the fibroblast and collagen produced to form excessive scar tissue, which is not as strong nor does it have contractile properties. Thus, even with proper care and sufficient recovery time, an athlete or an injured person may be re-injured upon return to normal activities due to the formation of scar tissue rather than muscle tissue. Therefore, besides taking preventive measures to facilitate healing, it is necessary to ascertain how therapeutic modalities such as low power laser affect non-collagenous and collagenous protein content of skeletal muscle

during its process of healing.

PROBLEM STATEMENT

A number of reports related to the clinical aspect of the use of Low Power Laser (LPL) Therapy (Mester et al. 1971, Kahn 1984, Mester et al. 1985 and Enwemeka 1988) are available. However, as mentioned by Fisher and associates (1990), the effects of therapeutic modalities commonly used in clinics on skeletal muscle at the cellular level following acute blunt trauma remain to be fully elucidated. Therefore, it is essential to physical therapists and related professionals to obtain information concerning the applicability and effectiveness of therapeutic modalities. In order to assess such information, an animal model (laboratory rat) and a standard injury device (Stratton et al. 1984) capable of reproducing a single impact injury that approximates a muscle contusion were used in this study.

The purpose of this investigation was to determine the effectiveness of a therapeutic dosage of laser and compare it with control groups in the treatment of acute blunt trauma to muscles. The measurements consisted of changes in non-collagenous and collagenous protein content of skeletal muscle tissue that take place at the cellular level.

RESEARCH HYPOTHESES

The following research hypotheses were investigated:

1 A single blunt trauma causes a decrease in the level of non-collagenous protein content in skeletal muscle of rats.

2 A single blunt trauma causes an increase in the level of collagenous protein content in skeletal muscle of rats.

3 Low power laser therapy applied to injured animal muscle increases the level of non-collagenous protein content compared to normal healing.

4 Low power laser therapy applied to injured animal muscle increases the level of collagenous protein content compared to normal healing.

5 Low power laser therapy applied to uninjured animal muscle has no effect on the level of non-collagenous protein content.

6 Low power laser therapy applied to uninjured animal muscle has no effect on the level of collagenous protein content.

DELIMITATIONS

This study was restricted to:

1 The use of an animal model.

2 One type of muscle trauma (acute blunt trauma) caused by an injury device that has been modified from Stratton and associates (1984) and approved by the Animal Welfare Committee of the University of Alberta.

3 The influence of one therapeutic modality (Low power laser) on the treatment of rat skeletal muscle (Medial Gastrocnemius) following acute blunt trauma.

LIMITATIONS

The limitations of this study included:

1 The use of one type of LPL equipment as specified in Chapter Three.

2 The use of Sprague Dawley strain of laboratory rats.

3 The use of specific biochemical techniques as described in Chapter Three to measure the non-collagenous and collagenous protein content of skeletal muscle of rats.

DEFINITIONS OF TERMS

I Low power laser

Laser is an acronym for the words " Light Amplification by Stimulated Emission of Radiation " (Calderhead and Ohshiro 1988). The wavelength of the laser is measured in nanometres and determines where a specific type of laser lies within the electromagnetic spectrum (10^{-4} - 10^{-6} m) and thus provide its classification. Lasers can be classified as high power or hot lasers, and low power or cold lasers (Calderhead and Ohshiro 1988).

In this study, a low power laser called Lazer - Train System IV was used because it was non-invasive, it had non-thermal therapeutic effects and has been used for tissue stimulation, while the high power laser has been used to cut or destroy tissue (Mester et al. 1985, Calderhead and Ohshiro 1988 and Gordon 1990).

2 Skeletal Muscle

Skeletal muscle is the tissue responsible for body movements and is composed of fibres which are bound together in bundles of different lengths and thickness (Woodburne 1988). These bundles of muscle can shorten or lengthen producing movements of the various parts of the body (Garret et al. 1988). In this study, skeletal muscle samples from the medial gastrocnemius were studied.

3 Animal Model

Twenty-eight male rats of the Sprague Dawley strain were used in this study. The animals weighed between 200 and 250 grams and were acquired from the Department of Biological Services of the University of Alberta.

4 Non-collagenous Protein Assay

The Lowry et al. (1951) biochemical technique of protein determination as described in Appendix A, was used to ascertain the non-collagenous protein content of the muscle samples used in this study.

5 Collagen Assay

The Woessner (1961) biochemical technique of collagen content determination described in Appendix B was used to ascertain the collagenous protein content of the muscle samples used in this study.

CHAPTER TWO

REVIEW OF THE LITERATURE

LOW POWER LASER THERAPY

INTRODUCTION

The Light Amplification by Stimulated Emission of Radiation or LASER had its first concepts developed by Einstein in 1916 as reported by Castel (1985). Initially, the term MASER was used meaning " Microwave Amplification by Stimulated Emission of Radiation ". The first study involving amplified electromagnetic radiation was performed using microwaves in 1950 by Townes and Schawlow as reported by Calderhead and Ohshiro (1988). These investigators showed it is possible to produce MASER beyond the optical region of the electromagnetic spectrum. Finally, as reported by Calderhead and Ohshiro (1988) in 1960, the term LASER was introduced by Theodore Maiman.

LASER CLASSIFICATION

Lasers can be generally classified as high power lasers or hot lasers (HPL) that have thermal effects and in low power lasers or cold lasers (LPL) that do not have thermal effects (Gordon 1990).

Since the 1960 's, as reported by Calderhead and Ohshiro (1988), low power laser has been used for treating various

clinical conditions such as open wounds (Kahn 1984) and bone fracture (Trelles and Mayayo 1987). According to Castel (1985), this therapeutic modality has been employed in Europe for more than a decade, but only recently has it emerged as a treatment device in North America. Positive results such as improvement of healing of chronic open injuries (Gogia et al. 1988) and increase in collagen synthesis (Mester and Jaszsagi-Nagi 1973) of the LPL therapy have been obtained in the treatment of wound healing and soft tissue injuries. Despite having no reported adverse effects or safety hazards, and having positive results reported to be associated with it, LPL therapy remains a questionable form of treatment (Basford 1989, Gordon 1990).

An example of a high power laser is the Carbon Dioxide laser which is used as a "light knife" in surgery to cut tissues and destroy tumours. Another example is the Argon laser which is used to spot weld detached retinas and vaporize dyes and coloured tissues in dermatology. Examples of LPL include the Krypton laser which is used in laser light shows and the Helium-Neon laser which is used for surveillance, alignment, price detection, and superficial tissue biostimulation. Another type of LPL more commonly seen and used as a therapeutic modality is the Gallium Arsenide laser which is used in sub-cutaneous tissue biostimulation. Therefore, in the biological sciences, the low power lasers are used for tissue stimulation whereas the high power lasers

are used primarily to destroy or cut tissue (Calderhead and Ohshiro 1988, Gordon 1990).

Even though lasers may be classified according to the intensity of energy delivered, their classification is usually based on the material used in the medium in which they are produced. Such materials might be crystal (ruby and neodymium, yttrium, aluminum and garnet in combination); gas (helium-neon, argon and carbon dioxide); semiconductor or diode (gallium-arsenide); liquid lasers or dye lasers or chemical lasers.

PHYSIOLOGICAL EFFECTS

Two investigations have reported on the usefulness of the LPL therapy in the treatment of a variety of conditions such as osteoarthritis and pain syndromes (Snyder - Mackler et al. 1986, Basford et al. 1987). Mester and associates (1985) reported that the LPL therapy enhanced the phagocytosis of leucocytes and accelerated wound healing in mice. Moreover, they demonstrated that LPL therapy was effective in accelerating wound healing in leg ulcers in clinical patients. In these patients, some changes that took place at the ulcer edge were considered by the investigators to be responsible for the positive results obtained. Some of their findings included: a) an increase in collagen ; b) reduction of cellular substance (this was not specified); c) an increase in epithelial activity and d) an increase in capillary formation.

The work of these investigators is said to be substantiated by their electron-microscopic observations, but such information is not available nor are the complete specifications of the parameters used. However, these changes, which reportedly enhanced the wound healing, were later confirmed by other studies (Cummings 1985, Enwemeka 1988). Despite all of the efforts made to ascertain the effectiveness of the LPL therapy, only a few studies have addressed how muscle responds to this relatively new therapeutic modality. Il'yasova and Popova (1980) reported and demonstrated histologically that the Helium-Neon (He-Ne) laser was capable of stimulating muscle repair and normalizing the process of regeneration of autografts that followed irradiation by X-rays, but the mechanism behind it was not clear and has not been determined. A large variety of low power lasers and techniques have been employed through the years of laser research, but the results have not been completely understood and there is a lack of completeness of information (Basford 1989). However, it is known that the biological effects of the LPL therapy are caused by the radiation proportioned by the laser (Castel 1985).

LASER ACTION AND COMPONENTS

Upon laser stimulation of the target tissue, there is a local reaction that is followed by a systemic response (Calderhead and Ohshiro 1988). The so called photothermal

reactions are the ones caused by the high power laser and happen above 40^0C (Calderhead and Ohshiro 1988). Such reactions may be used to cause coagulation, vaporization, and to cut the target tissue. As these reactions were not the objectives of this study, they will not be discussed further. However, there are other local reactions which are non-photothermal (up to 36^0C) and are caused by the low power laser (Calderhead and Ohshiro 1988). Therefore, since there is no heating effect when LPL therapy is applied, its induced reactions are classified as athermal. Such information is important when the effects of LPL therapy are being assessed at the cellular level, because at this level there is a varied response of different cells to laser therapy with temperature variations (Calderhead and Ohshiro 1988).

The physical phenomena responsible for the creation of laser energy is better understood when described along with the laser components. Lasers need a medium to be developed and require a power supply, usually electrical, that introduces energy into the medium (Fig.1), (Castel 1985, Calderhead and Ohshiro 1988). When energy is introduced into the laser medium, it is converted into light or photon energy (Fig.2). The laser medium may be composed of either solid, liquid or gaseous substances that are comprised of atoms which are bound together to form molecules. The laser medium is composed of atoms which are composed of electrons. When energy is pumped into the medium (Fig.3) which contains atoms,

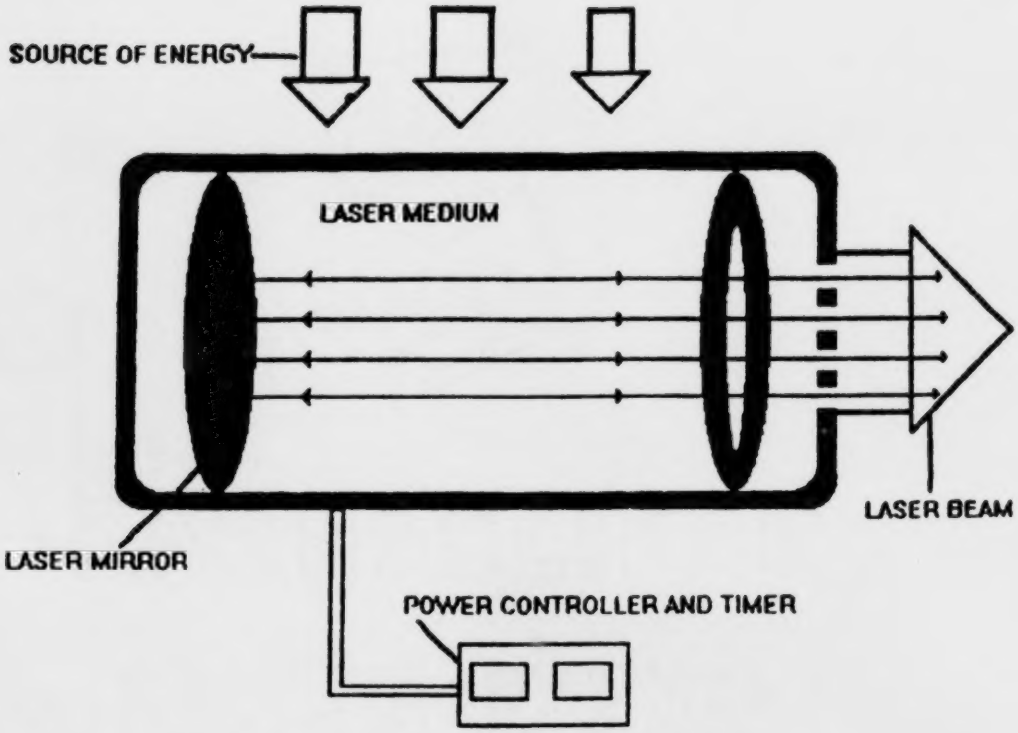

FIGURE 1: LASER SYSTEM WITH ITS PARTS

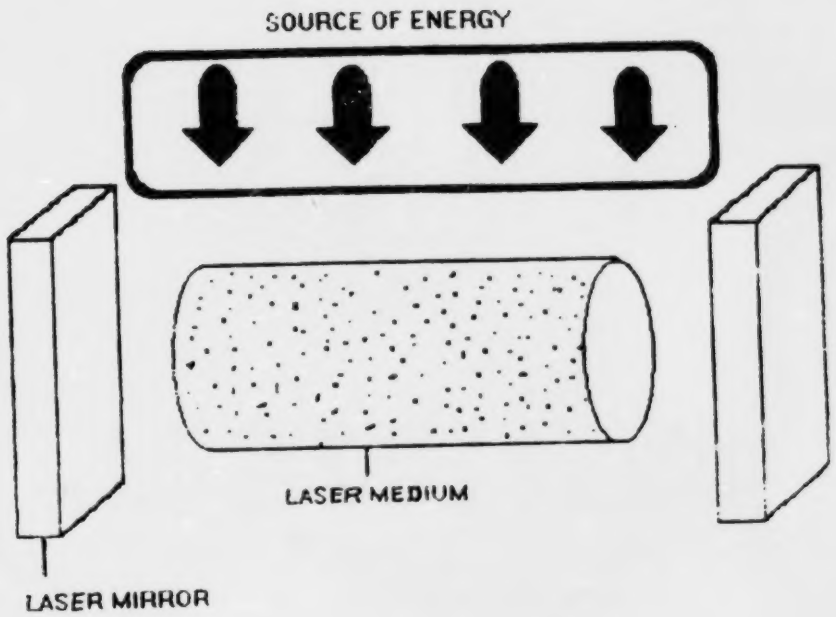

FIGURE 2: INTRODUCTION OF ENERGY INTO THE LASER MEDIUM

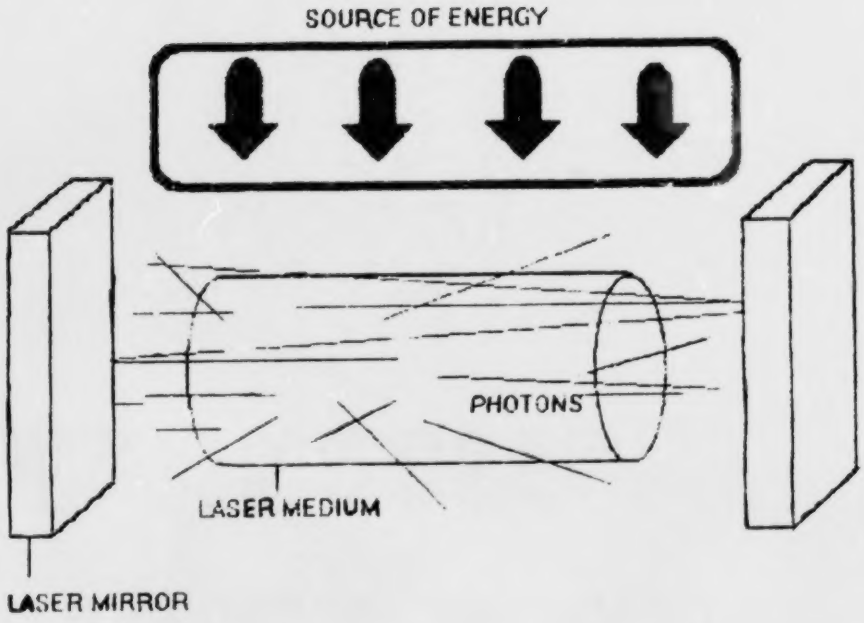

FIGURE 3: RANDOM PHOTON EMISSION FOLLOWING PUMPING OF ENERGY INTO THE MEDIUM

there are some changes in the atoms as their electrons absorb energy. The atoms absorb energy as their outer electrons are hit by photons which cause the atoms to become excited and jump to an outer orbit of higher energy. However, atoms stay in this excited state only temporarily and then spontaneously return to their normal energy level by releasing the extra energy that had been absorbed in the form of a photon. The photon is considered light energy and its wavelength specificity is related to its medium. When one photon, which has been spontaneously released, strikes an atom that is in its excited state there is release of energy. Such energy is called stimulated emission of radiation (Calderhead and Ohshiro 1988). Another laser component is the resonating cavity which contains the laser medium. Such a cavity is usually composed of two pieces of silver coated glass or mirrors (Fig.1). One of the mirrors is only partially coated to allow part of the laser light to escape. This quality enables the mirrors to reflect the laser differently. The process of laser creation occurs quickly and at random (Fig.3). Initially, most of the photons are lost through the walls of the resonating cavity. As the photons emitted by stimulated emission are reflected back into the cavity, they stimulate the emission of more photons up to the point where there will be more excited atoms than resting atoms. This phenomenum is called population inversion. As more photons. are released, an intense photon resonance is built up inside

the cavity (Fig.4) and these photons stimulate the release of more photons and with it, the light being produced becomes stronger or amplified (Fig.5). Therefore, the process is called Light Amplification by Stimulated Emission of Radiation or LASER.

LASER CHARACTERISTICS

Laser radiation or laser light and ordinary light have different physical aspects. In the vast electromagnetic spectrum, they have different wavelengths. Wavelength is the distance from peak to peak along a waveform and it is measured in nanometres (Nm) (Fig.6). The wavelength determines whether the beam of light produced by the source is visible or invisible and its colour if visible. A laser emits energy at a specific wavelength, has specific colour and therefore, it is monochromatic. The photons are emitted in only one direction and travel back and forth between the mirrors of the resonating cavity (Fig.1). Therefore, they arrive at their target in phase and because of this, the emerging laser beam has only minimal divergence and has its energy focused. This property is called collimation (Calderhead and Ohshiro 1988) and the sum of monochromaticity, phase and collimation is called coherence (Fig.7). These three characteristics of the laser light enable the differentiation between laser and other forms of radiant energy.

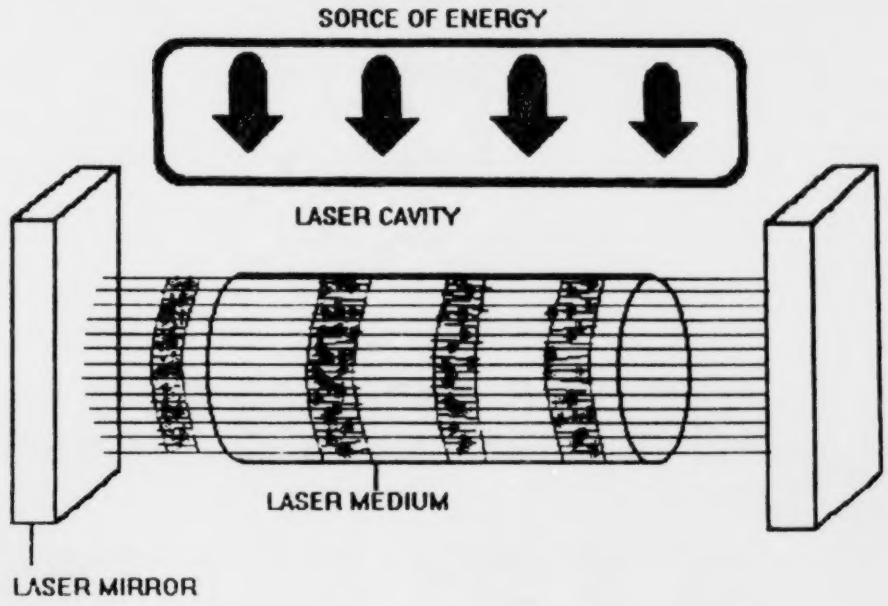

FIGURE 4: BUILD UP OF PHOTON RESONANCE

INSIDE THE LASER CAVITY

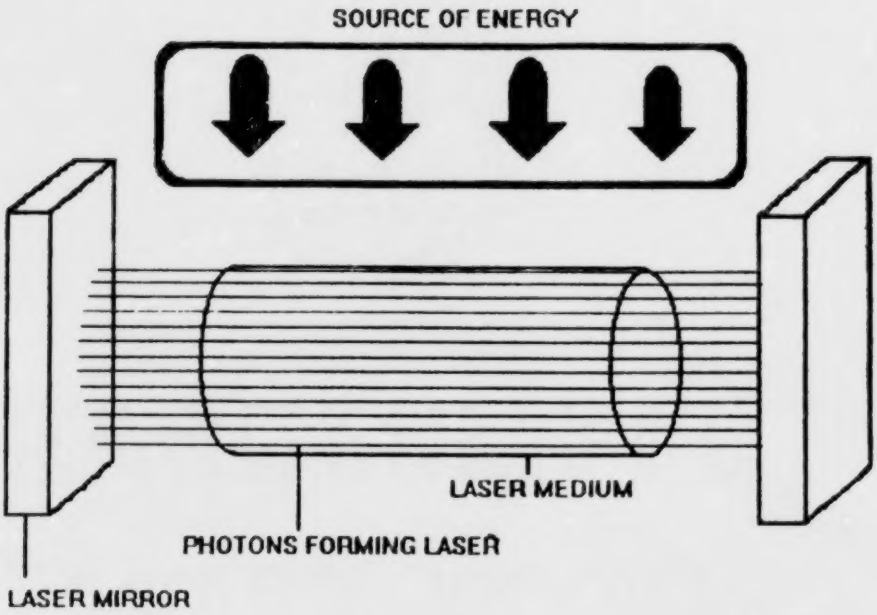

FIGURE 5: PHOTONS BUILD UP BECOMING INTENSE AND FORMING THE LASER

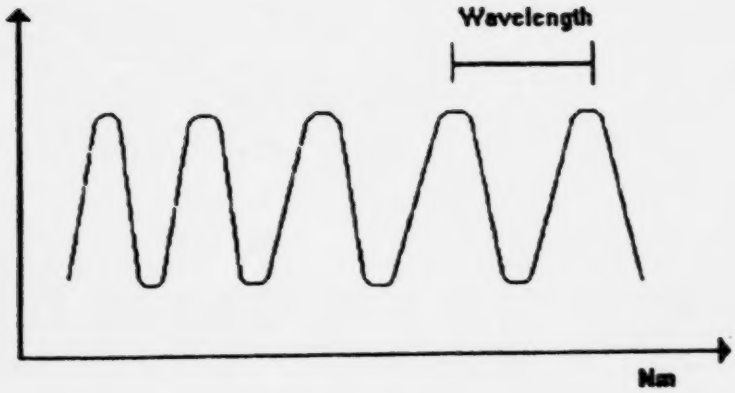

FIGURE 6: REPRESENTATION OF LASER WAVELENGTH

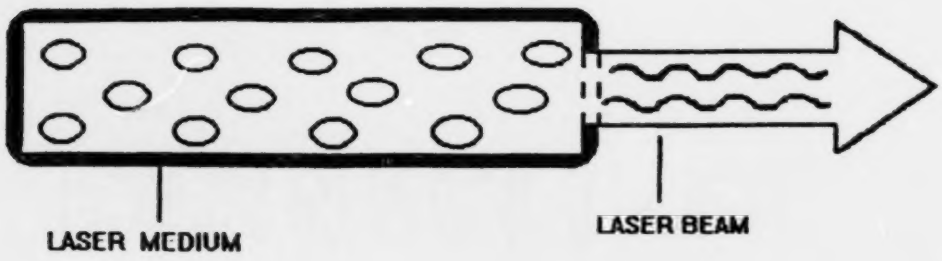

FIGURE 7: LASER COHERENCE

The produced laser beam may be delivered to the target tissue directly or by means of a waveguide. When delivered directly, the laser medium is contained in the probe used for treatment and the laser beam does not lose its coherence. When delivered through a waveguide, the type used is dependent on the wavelength of the laser. Due to its flexibility and ease of operation, the flexible quartz fibre optic is a popular delivery system (Calderhead and Ohshiro 1988). This system however is not totally coherent because there is a variation in the pace of the light beam during its transmission through the fibre optic device, but this system still has monochromaticity and can be well focused (Calderhead and Ohshiro 1988).

Presently, most lasers can produce stimulation either in the continuous or the pulsed mode. An example of a commonly used laser that is used to stimulate wound healing (Kana 1981) in the continuous mode is the He-Ne gas laser with a wavelength of 632.8 Nm. An example of a pulsed laser is the Gallium Arsenide (Ga As) diode laser with a wavelength of 904 Nm (Castel 1985).

Once the laser energy is produced and delivered to a target tissue, there is interaction between the tissue and the laser. Four basic interactions have been described: Reflection, Transmission, Scattering and Absorption (Fig.8). Only absorption is discussed as it applies directly to the present study. Absorption is the reaction one wants to achieve

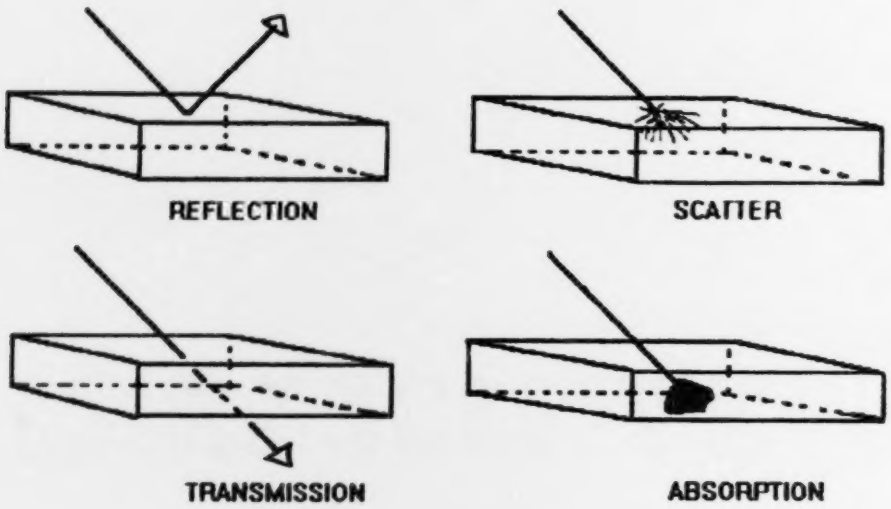

FIGURE 8: POSSIBLE LASER - TISSUE INTERACTIONS

in order to obtain a physiological effect (Lynn and Seitz 1990). Upon absorption, there is transfer of energy from incident photons to the absorbing molecules within the tissues. According to the wavelength and power of the laser beam, such absorption may lead to either a thermal or a non thermal reaction (Gordon 1990). Visible light lasers have wavelengths between 400 and 700 Nm. It is known that visible light laser is pigment specific, that is, when a pigment is added to a non-pigmented area, the absorption reaction increases. Moreover, each visible light laser is better absorbed by its complimentary colour. Visible light lasers penetrate less than the infrared light lasers (Parrish 1981). In terms of absorption, it is known that near infrared wavelengths of 700 to 1600 Nm are absorbed in biologic protein and the far infrared wavelength of co_2 laser at 10600 Nm is probably completely absorbed in water molecules (Lynn and Seitz 1990). For this reason, this type of laser is absorbed in the first layers of the target tissue where there is a higher proportion of water molecules (Calderhead and Ohshiro 1988).

Laser energy is based on its photobiothermal reactions and such reactions are temperature specific. The application of laser in physical therapy is based on its non-thermal reactions, because the aim is mainly to obtain tissue stimulation and, according to the literature, this stimulation may lead to physiological effects such as an increase in the

synthesis of collagen (Lyons et al. 1987), reduction of pain (Snyder-Mackler et al. 1986), increase in the rate of wound healing (Enwemeka 1988) and increase in muscle fiber regeneration (Mester et al. 1975 and Il'yasova and Popova 1980).

LASER EXPERIMENTS

Most of the initial experiments on LPL therapy were not controlled. Moreover, they did not demonstrate reliable methodology, had incomplete descriptions and limited blinding techniques (Basford 1989). For these reasons, the results of such studies could be considered to be based on empirical observations and did not completely ascertain the beneficial effects of the LPL therapy (Basford 1989, Gordon 1990). However, as the popularity and consequent demand for the application of low power laser therapy increased, the results of later research on this modality appeared to become more reliable (Basford 1989).

According to Basford (1989), the LPL therapy research can be divided into three areas: A)cellular function; B)animal studies, and C)human studies. Research at the level of cellular function is considered to be the most developed of the three areas and may support the evidence of biological effects caused by LPL therapy (Basford 1989). The effects of LPL therapy upon collagenous protein synthesis (Mester et al. 1985, Lyons et al. 1987), non-collagenous protein synthesis,

and phagocytosis (Mester et al. 1985) were investigated among other cell studies carried out to evaluate the effectiveness and value of the LPL therapy.

In 1985, Mester and associates published a paper in which the authors' experimental and clinical use of Low power laser therapy (LPLT) on fifteen biological systems over a period of twenty years was summarized. At the cell level these authors concluded that LPLT (defined as energy density delivered between 1 and 4 j/cm^2) had a stimulating effect and high power laser therapy (defined as energy density delivered above $4j/cm^2$) had an inhibiting effect. An example of a stimulating effect of LPLT at the cell level cited by these authors was the increase in phagocytosis of leucocytes proportioned by a ruby laser at a dosage of 0.05 j/cm^2 and a decrease or inhibition of the phagocytosis at dosages of 2 to 4 j/cm^2, which is not completely in accordance with their dosage parameters.

Other investigators (Pourreau-Schneider et al. 1990) showed that upon LPLT, in vitro, fibroblastic cells may differentiate into myofibroblastic cells. These authors used transmission electronmicrocospy and immunohistochemistry to quantitate such effects. Cell differentiation in cultures was observed 24 hours after exposure to HeNe laser irradiation in contrast to controls that were only comprised of resting and active fibroblasts. Using this information, these authors studied the effects of LPLT upon in situ wounded gums

following wisdom tooth extraction. Myofibroblasts were found 48 hours post treatment in laser treated samples, but not in untreated control samples. The relevance of these findings related to the fact that such induction in myofibroblasts formation might be analogous to that which occurs in vivo and might be of clinical significance in the acceleration of the wound healing process (Pourreau-Schneider et al. 1990).

Animal studies have shown positive results in soft tissue wound healing (Kana 1981 and Lyons et al. 1987). According to Mester and Jaszsagi-Nagy (1973), LPLT (ruby laser) was effective on wound healing when applied in the early stages of repair. This effect was shown when 4 j/cm^2 were applied to cicatricial tissue following an artificial incision. The results indicated that the collagen synthesis of treated animals exceeded that of controls by 30 to 50%. When the animals were treated at 0 and 48 hours post-injury and sacrificed at 3 and 5 days, there was a significant difference between control and laser treated wounds ($p < .001$).

Based on clinical observations that LPLT may stimulate wound healing, Lyons and associates (1987) examined the effects of a HeNe laser at 1.22 j/cm^2 of energy density upon experimental wounds in mice, in vivo. Biochemical analysis indicated that the collagen concentration increased at two and four weeks in laser treated animals in comparison to untreated controls. Although the level of significance was not mentioned for this comparison, in the same study, tensile

strength of the wounds was determined and in the treated animals, it was found to be significantly greater than the controls ($P<.001$) at one and two weeks, which was in agreement with the concentration of collagen found.

In an earlier animal study, Kana (1981) found HeNe laser at an energy density of 4 j/cm^2 to have a maximum effect on collagen synthesis in the healing of open skin wounds of rats as compared to energy densities of 10 and 20 j/cm^2 ($p < .01$). Between days 3 and 12, wound closure of laser treated rats was found to be significantly better than that found for untreated controls ($p < .02$ and $p < .005$ respectively).

Two studies (Mester et al. 1975 and Il'yasova and Popova 1980) reported the effects of LPLT upon muscle tissue repair using animal models. As mentioned previously, Il'yasova and Popova (1980) found HeNe capable of stimulating skeletal muscle tissue repair following transplantation and radiation by x-rays. The authors studied whether the muscle recovered from its structural changes which occurred after transplantation due to a more rapid elimination of the muscle cells damaged by the x-rays or if there was any stimulation toward the intracellular repair in the muscle which had possibly been damaged by radiation. Thus, LPLT was used to observe if the consequences of the trauma caused by the x-rays could be eliminated and the muscle reacquire its ability to regenerate. LPLT was found to be effective when given for ten days before transplantation, through histological observations

after two weeks, one and two months. However, there were no statistical results presented by the authors and the laser utilized has a low depth of penetration. Moreover, these authors referred to an international bibliography which was not in English and unavailable at the time of this study.

In an earlier investigation, Mester and associates (1975) studied the process of skeletal muscle regeneration on experimental models following injury by incisions. LPLT (ruby laser) was used at 1 j/cm^2 four times every third day. Histology was used to assess the effects of LPLT on different days post injury. It was concluded that LPLT not only accelerated the process of wound healing induced, but also positively influenced the regeneration of muscle fibers following the injury. Furthermore, the authors also noted that an adverse result due to a summation of repeated radiation effects might occur. Such adverse effects were related to the promotion of excess of connective tissue that interfered with the regenerative capacity of muscle fibers. The statistical results were not reported in the paper.

Human studies have shown positive results (Shiroto et al. 1986), but such results might not be of significance if they are based on uncontrolled procedures. In more controlled studies, the benefits of LPL therapy in humans have been partly ascertained by some authors (Emmanouilidis and Diamantopolous 1986, Walker et al. 1986). For example, in a controlled double blind study of the LPL therapy in

professional athletes, Emmanouilidis and Diamantopoulous (1986) used a continuous wave infrared low power laser of 820 nm wavelength to accelerate the rehabilitation process of chronic pain relief of athletic injuries. Two groups were selected and matched according to pain, history, pathology and sports activity. LPLT was applied daily to pain trigger points. In comparison to the placebo group, athletes in the laser treated group had a faster return to training with pain relief. Although no statistical results were presented, it was concluded by the authors that this modality should be recommended due to its positive results upon pain relief.

In a controlled study (Walker et al. 1986), LPLT (HeNe laser, 1 mW) to subjects with rheumatoid arthritis was compared to control placebo subjects. Analysis of variance with repeated measures on pain ratings, activity levels and drug use indicated that the treatment group had a significant decrease in pain ratings ($p < .005$) and increase in activity levels ($p < .005$). No significant difference was found in drug intake. Therefore, in view of these results, LPLT may be of significance in the management of patients with rheumatoid arthritis.

However, despite of the above shown findings, other authors have not found or confirmed the benefits of this modality (Mcauley and Ysla 1985, Basford et al. 1987). In a controlled study (Basford et al. 1987), the effects of the HeNe laser of 632.8 nm on the thumb of osteoarthritic patients

were investigated. Only a slight decrease in tenderness of the treated metacarpophalangeal and interphalangeal joint was found ($p < .01$ and .05 respectively). A small increase in three finger chuck pinch strength ($p < .04$) was found between the first and last session of the laser treated group. However, other measurements such as range of motion, pain, grip and pinch strength and activity level did not differ significantly. According to these authors, the dosage applied (15 seconds irradiations to four spots, four times at 0.9 mW continuous wave HeNe laser, penetrated the skin and transluminated the fingers, but it was believed that some energy was delivered to the target tissue (Basford et al. 1987). Therefore, It was concluded that this form of therapy was not effective in the cases studied.

In a controlled study by Mcauley and Ysla (1987), the results indicated that LPLT had no therapeutic benefit upon pain in patients suffering from distal median compression neuropathy associated with carpal tunnel syndrome.

Due to the fact that there is a great variation in the parameters used in different investigations, it is difficult to compare the results obtained. Therefore, future studies should be aimed at standardization of the parameters to be used (Basford 1989 and Gordon 1990).

MUSCLE HEALING

INTRODUCTION

It is known that skeletal muscle tissue has the capability of regenerating following injuries. Such capability is described in another section of this review. However, the end result of the process of regeneration is dependent upon factors such as the intensity, duration and type of insult to the muscle involved. In sports, athletes are subject to different types of injury. For example, muscle strains are indirect injuries related to excessive stretch or tension. A muscle contusion may be caused by a direct blow to the limb and constitutes one of the most common soft tissue injuries seen among athletes (Garret et al. 1988). A muscle contusion may lead to pain, and disability followed by periods of rehabilitation, in which physical therapy care is important.

Immediately after a muscle is contused or strained, a local inflammatory response is established. Such an inflammatory response has been described by Gould (1990) and is part of the muscle healing process that follows an injury.

MUSCLE REGENERATION AND THE INFLAMMATORY RESPONSE

According to Allbrook (1973), in every millimeter (mm) of a muscle fibre, there are one hundred muscle nuclei. He estimated that 95% of these nuclei lie inside the cell

membrane, while 5% lie outside and belong to satellite cells. Satellite cells are unspecialized and mononucleated cells located between the muscle fibre and its enveloping basal lamina. Satellite cells do not have myofilaments, but are capable of undergoing mitotic division and thus, they increase the number of muscle nuclei available. Allbrook (1973) reported that these cells were used as the supply cells when there was muscle damage. Upon muscle damage, satellite cells are considered to be the source of nuclei for the new growing fibres. These cells undergo mitotic division providing for the growth of new fibres. Besides being capable of migrating to the area of muscle damage and undergoing mitotic division to provide for the growth of new fibres, these cells continue to divide and add to the further maintenance of the peripheral cell population.

Myoblasts are muscle cells capable of producing contractile protein and thus contributing to the formation of muscle fibres (Carlson and Faulkner 1983). After injury, satellite cells undergo mitotic division and the resultant daughter cells may become active myoblasts. These myoblasts may fuse to form myotubes which are elongated and multinucleated cells. Once myotubes are formed, they are responsible for synthesizing and assembling contractile proteins in arranged arrays of myofilaments. Collagenous protein has to be present in order for myotubes to be formed, because collagen forms a sheath around fusing myoblasts during

myotube formation (Allbrook 1973). Therefore, myoblasts and fibroblast are associated and under the right conditions of nutrition, muscle fibres may regenerate. Initially, the process of assembling contractile proteins is started in the centre of the myotube, but as the myotube matures, the nuclei become more compact and migrate to the periphery. At this point, the myotube is called a muscle fibre with its neuromuscular junction which has also been formed (Engel and Banker 1986).

Various cellular organelles may become involved in the process of muscle regeneration. For example, in mature muscle fibres, the muscle nuclei occupy a peripheral position beneath the sarcolemmal membrane, but during regeneration, the muscle nuclei migrate to the centre of the fibre and the sarcoplasm becomes rich in rough endoplasmatic reticulum, golgi complexes and ribosomes. As the muscle fibres mature, the satellite cells decrease their myogenic activity and resume their original position on the periphery (Engel and Banker 1986).

According to Gould (1990), the inflammatory response is basically the same in the whole body, regardless of the nature of the responsible agent that causes it. With an inflammatory response, there are chemical, vascular and metabolic changes that are followed by a process of repair (Reed and Zarro 1990). Direct acute blunt trauma to muscle, as in muscle contusion, results in direct damage to the cells of the muscle tissue. Moreover, associated nerves and blood

vessels may be affected by direct trauma. Upon muscle contusion, blood vessels may be torn and allow blood extravasation into the interstitial space of the injured area. There is also an increase in the permeability of the nondisruptured vessels in the injured area. Such an increase in the permeability allows the passage of plasm proteins, colloids, and water into the interstitial spaces, resulting in swelling or edema. The vascular tissues then act to mobilize and transport white blood cells, which are the defense component of the blood, to the injured area. These cells move to the margin of the capillaries and adhere to the wall of the damaged capillary in a process called margination (Allbrook et al. 1966). Some of these cells then migrate through the walls of the vessels in a process called diapedesis and move to the injury site apparently on the basis of chemical attraction. Finally, the white blood cells, initially neutrophils and later monocytes which will transform into macrophages, invade the injured area, and are responsible for breakdown and removal of the remains of damaged muscle fibres that originated from the muscle contusion. This process is called phagocytosis (Gould 1990). In order for phagocytosis to occur, proper vascularization is important as most of the phagocytic cells originate from the blood.

As the removal of the damaged muscle fibre is being accomplished, myoblast cells originating either from the myonuclei of the cells that were left or from satellite cells,

are involved in the regeneration of new muscle fibres (Carlson and Faulkner 1983). For regeneration to be effective, it is important that the basal lamina be intact and not affected by the injury, thus keeping the infiltration of fibroblasts away and providing a favourable environment for regeneration. Once myoblasts are established, they fuse into myotubes that differentiate into mature muscle fibres with the peripheral nuclei as in normal skeletal muscle fibres (Carlson and Faulkner 1983).

CHANGES IN PROTEIN IN SKELETAL MUSCLE

In skeletal muscles, contraction is the result of the sliding of actin over myosin microfilaments present in the sarcoplasm of the muscle fibres. Proteins are the major constituents of muscle fibres. However, proteins undergo continuous breakdown and synthesis of new protein is necessary to replace them (Rodemann and Goldberg 1982, Tischler and Fagan 1983).

Both protein breakdown and synthesis in skeletal muscle need to be regulated and the levels of protein may be determined by the net balance between the rates of protein breakdown (degradation) and the rates of protein synthesis (regeneration). According to Tischler and Fagan (1983), following a muscle contusion, there is an increase in muscle protein catabolism, which is responsible for a net decrease in the muscle protein content of the injured muscle. This

decrease is followed by an increase in muscle protein synthesis.

In another study by Fisher and associates (1990), skeletal muscles were subjected to acute blunt trauma and muscle protein breakdown and synthesis were measured. It was found that in the 48 hours following trauma, there was a significant catabolic response with a decrease in the total protein content. Moreover, muscle protein synthesis started 72 hours after trauma, but complete muscle protein repletion did not take place until 21 days post-injury.

The causes of such changes in muscle metabolism as observed by protein synthesis and breakdown , are not completely known (Tischler and Fagan 1982 and 1983). Therefore, future studies should be aimed to investigate such causes. Moreover, it is important for sports therapists to study and assess what and how currently employed therapeutic modalities may affect muscle metabolism.

CONNECTIVE TISSUE OF SKELETAL MUSCLE

Skeletal muscles have a variety of morphological forms and modes of action. Nevertheless, all have the same basic structure, being composed of extremely elongated multinucleated cells, which are designated as muscle fibres (Allbrook 1973). Whole muscles are composed of bundles of individual muscle fibres that are bound together by connective tissue. This connective tissue is part of the extracellular

matrix of skeletal muscle.

Individual muscle fibres are grouped together into elongated bundles called fasciculi with delicate connective tissue called endomysium investing each fibre. The investing endomysium is composed of fibres of collagen, reticulin and elastin. The fasciculi are surrounded by loose collagenous connective tissue called perimysium. Most muscles consist of many fasciculi and the whole muscle mass is invested in a dense, outer connective tissue sheath called epimysium (Woodburne 1988). The size of the fasciculi reflects the function of the particular muscle concerned. Within each muscle, the connective tissue component contains both collagen and elastic fibres acting as a flexible skeleton to which individual muscle fibres are anchored, thus providing mechanical support to the muscle. Moreover, this connective tissue may aid in the control Mw the cellular metabolism of the muscle fibre (Engel and Banker 1986).

Following muscle injury, two processes are known to compete. The regeneration of the damaged muscle and the production of connective tissue scar (Lehto et al. 1985). The formation of excessive connective tissue scar may lead to the formation of a mechanical barrier to the regenerating muscle fibres and therefore inhibit the process of muscle regeneration.

In cases where large volumes of muscle have to regenerate, fibroblast and collagen comprising fibrous scar

tissue are laid down before myoblasts and muscle fibres, because the former are formed more rapidly than the latter and constitute a barrier to the proliferation of the latter. In addition, some connective tissue is necessary for the process of muscle regeneration, because it forms a sheath around fusing myoblasts during myotube formation (Allbrook 1973).

A number of cells have been listed as capable of synthesizing collagen (Lehto et al. 1986). Fibroblasts are considered specialized cells capable of repairing perimysial and endomysial connective tissue and therefore capable of repairing connective tissue affected by trauma.

In the healing of skeletal muscle injuries, there is stimulation of cell proliferation and of synthesis of extracellular matrix components. During the process of formation of granulation tissue, the synthesis of collagen is enhanced by an increase in the number of fibroblasts in the wounded area. However, the synthesis of collagen is gradually reduced after an acute inflammatory reaction is completed. It is believed that such synthesis persists if the inflammatory reaction is not terminated and therefore excessive scar tissue may result. Gay and associates (1978) reported that the synthesis of collagen during wound healing may be started even before any active fibroblasts are found in the wounded area. Therefore, one may infer that collagen might also be synthesized by other types of cells in the region.

During this healing process of injured muscles, while

granulation tissue is being formed, collagen is being organized into bundles. As healing progresses, the newly formed granulation tissue covers the injured area and scar connective tissue production is completed. Studying the scar formation after skeletal muscle injury, Lehto and associates (1986), found that synthesis of extracellular connective tissue components started two days after trauma, was intensive between days 5 and 21 and decreased in the following weeks. Moreover, This study showed that not only the rate of collagen synthesis within cells decreased during the process of healing, but also the rate of other protein synthesized.

Even though clinical studies have been done to report on the efficacy of the therapeutic modalities used for treating muscles, only a few experimental studies have examined cellular events and their consequences in muscle injuries. Therefore, in the present study, the collagen content of the sampled muscles was determined by the Woessner method (1961), in which hydroxyproline is used as an indicator of the presence of the metabolism of collagen as described in appendix B. The non-collagenous protein content was determined by the method developed by Lowry et al. (1951) as described in appendix A.

CHARACTERISTICS OF EXPERIMENTAL MODELS OF ACUTE BLUNT TRAUMA TO SKELETAL MUSCLE

Experimental models to study muscle trauma have been

reported (Allbrook et al. 1966, Jarvinen and Sorvati 1975, Tischler and Fagan 1983). However, none of these authors fully reproduced an injury that could be representative of an acute blunt trauma that takes place in sports. Jarvinen and Sorvati (1975) produced muscle trauma by inducing a crushing injury without tearing of the skin. Crushing closely reproduces what happens to traumatized muscle in contact sports. These authors claimed that their crushing technique was constant. However, even though measures of location, dimension and breaking strength of the injured muscle were performed, no measurements were taken at the cellular level.

Tischler and Fagan (1983) used a reflex hammer device to deliver multiple light blows to muscles over a period of time. According to the histochemical and biochemical measurements performed, it was concluded that such a method was capable of inducing muscle damage. However, one must consider that such a method, in which multiple light blows were used, may not be representative of the type of muscle injuries that occur in sports.

Well before Tischler and Fagan (1983), Allbrook and associates (1966) studied muscle injury and regeneration by using a single blow to the lower limb. However, in this study, the applied force not only caused damage to the soft tissues, but also fractured the fibula of the animal. Thus, such a device and method differ and are not representative of the type of muscle injury of this study.

Other methods such as induced ischemia and induced exercise injury, have been used to study muscle injury. However, as mentioned before none of these methods agreed with the objectives of this study.

In sports as well as in accidental injuries, there is great variability of injuries, in the sense that there is variation in the forces applied,and in the area and compliance of tissues being hit. Therefore, in order to study acute blunt trauma to skeletal muscle, an injury device capable of delivering a standard injury, had to be designed. Stratton and associates (1984) developed an injury device which was capable of causing a controlled and reproducible impact trauma that approximated the acute blunt trauma seen in sports. Their device had the advantage of producing a single impact blow, which is common in sports, and produced an injury in which there was no tearing of the skin. This latter aspect was an advantage in the sense that a tear could complicate and delay the healing process due to a possibility of resulting infection.

CHAPTER THREE

METHODOLOGY

INTRODUCTION

Nerve function problems and rheumatoid arthritis have received more attention concerning LPL therapy research than soft tissue injuries (Basford 1989) which constitute a greater problem among athletes. Among the soft tissue injuries, acute muscle injury or muscle trauma caused by a direct blow or impact is very common and the mechanism of muscle repair that takes place after the injury remains unclear.

The mechanism of action behind LPL therapy might be related to the effects of its light. Each distinct laser has a different wavelength in the electromagnetic spectrum (EMS). At the lower end of the EMS, the laser light penetrates less. Therefore, the photobioactivation caused by LPL therapy is wavelength dependent. That is, the effects of laser are related to how much it can penetrate and stimulate the tissues. However, there is need to investigate how laser is better than ordinary light energy, which does not penetrate skin and the underlying tissues. Currently, there are laser systems available either in the invisible infrared or in the visible red spectrum. The helium neon laser (HeNe) is a monochromatic, visible red laser with a wavelength of 632.8 Nm. It is reported to penetrate up to 0.7 cm directly and up to 1.0 cm indirectly in human tissue (Castel 1985). As the greater part of the energy delivered by this laser is absorbed

by the skin, it is considered to have only a superficial action on human tissues. An example of invisible infrared laser is the galium arsenide (GaAs) with a wavelength of 904 Nm. This laser is reported to penetrate up to 4.0 cm directly and up to 8.0 cm indirectly (Castel 1985). Therefore, considering that in this study, the effectiveness of LPL therapy was evaluated in treating muscle injuries, this type of laser was applied. Therefore, one of the objectives of this study was to assess the effectiveness of this modality. Due to the invasive characteristics involved in the data collection of this study, which involved the necessity of inducing a standardized acute blunt trauma to skeletal muscle and obtaining muscle tissue sample from the subjects, an animal model was used.

SUBJECTS

This study required the use of laboratory rats (Sprague Dawley strain) and all the procedures carried out were in accordance with the guiding principles for the care and use of laboratory animals of the Canadian Council on Animal Care (CCAC) which was published in 1988. Twenty-Eight male rats were used. The animals initial body weight ranged from 230 to 260 grams. The animals were weighed and housed in twelve standard wire cages in groups of two or three of approximately the same weight to decrease the chances of conflict among them. Commercial Rat Chow and water were provided ad libitum.

The animals were kept on a 12 hour light to 12 hour dark cycle as this cycle is reported to be the normal rat cycle (Laboratory rats 1988). The animals were also weighed on the last day of the experiment to see if there was any significant difference in their body weight which could be related to the injury or LPLT used in this study.

EXPERIMENTAL TRAUMA

The effects of laser therapy following a single impact trauma upon muscle were studied. The animals used were subjected to an experimentally induced single impact trauma to the medial aspect of the gastrocnemius muscle of the right limb. The experimental trauma was delivered by an injury device (Fig.9) which was a modification developed by Fisher et al. (1990) of the injury apparatus originally developed by Stratton and associates (1984). Following C.C.A.C procedures, all animals to suffer trauma were briefly anaesthetized with halothane. In addition, all other animals used in the study were also briefly anaesthetized to control for any possible effect of the anaesthetic used. The animals were held against the flow of the halothane for approximately forty five seconds to minimize any pain (masking technique).

The injury device was placed over the area to be injured. The exact location of the muscle to be traumatized was determined through palpation of its large belly. In order to prevent any abrasion or tearing of the skin, the spot to be

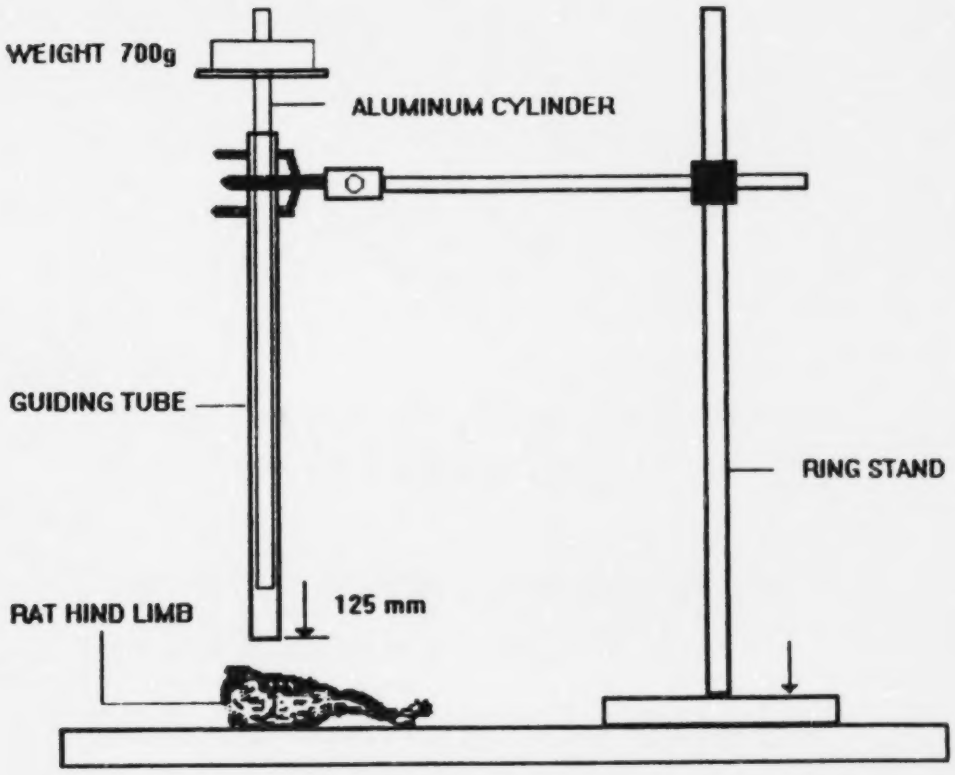

FIGURE 9: INJURY DEVICE

traumatized was padded with a 0.5 cm layer of gauze. The rat tibia was protected from fracturing by positioning it out of reach of the falling cylinder and the foot was kept at a 90 degree angle to the tibia (Fisher et al. 1990). Trauma was caused by having a solid aluminum cylinder of 700 grams dropped once over the padded muscle of the right limb. The cylinder was 1.38 cm in diameter and 27 cm long. The cylinder was dropped through a distance of 125 mm onto the muscle. The rat limb was positioned manually In order to observe and ensure the localization of the injury site, prior to trauma the trauma spot was marked with indelible ink. The cylinder was dropped through a tubular guide which was fixed to a ring stand in order to provide a better stabilization of the apparatus and accuracy of the force delivered. The force delivered by the falling cylinder was calculated to be 0.57 Newton-Meters per Square Centimeters (Nm/Cm^2), using the following formula: F = E/A where F is the force; E is the Kinetic energy, and A is the area of the piston.

LASER THERAPY

Castel (1985) reported that in order to obtain physiologic effects, when applying the LPL therapy, the total energy delivered is the important therapeutic parameter to be varied. Castel (1985) stated that by altering the frequency, no significant benefit is really achieved. According to Enwemeka (1988) the dosage, frequency of treatment, and depth

of penetration are still not clear. The use of LPL therapy on humans causes absorption and dispersion of the laser light that varies according to the composition of the tissue irradiated. Kleinkort and Russel (1984) observed that lasers have a variety of physiologic effects, but all are dependent upon the energy density delivered, wavelength, and exposure time. Based in experiments reported by various authors, Wooley-Hart (1988) suggested that each user was able to develop his/her own calculations and that considerations about the parameters to be used should be done according to the objectives to be pursued. Therefore, in view of the objectives of this investigation, the infrared GaAs low power laser was used. The animals received 1 J/Cm^2 of energy density through a probe tip of 0.01 Cm^2. The laser unit was turned on for approximately 11.1 seconds for each the two spots treated in order to deliver the wanted energy and achieve the biostimulative effects in deep tissue as suggested in the literature (Castel 1985, Mester et al. 1975).

The treatment commenced at day 3 following trauma and was given daily up to and including day 12 on a once per day basis, at the same time each day. According to other investigators (Tischler and Fagan 1983), acute blunt trauma may be followed by muscle protein wasting or loss. Such loss has been observed in the first three days following trauma and thereafter, there should be muscle protein repletion (Fisher et al. 1990). In this study, low power laser therapy was

applied from day 3 up to day 12. The period of treatment chosen for this study was selected to represent a period in which muscle is regenerating and therefore variations in non-collagenous and collagenous protein content could be more noticeable and measurable.

The laser equipment used was the Lazer-Train Unit (Stern Equipment) which is an infrared Gallium-Arsenide pulsed laser with a wavelength of 904 Nm. It had an average power output of 0.9 mW. According to the formula **E = W x Sec / A** (**E** is the energy density in joules per square centimeters, **W** is the average power in watts, **Sec** is the time in seconds and **A** is the radiation area in square centimeters). In one second, this unit could deliver 0.09 J/Cm^2 of energy to the area under the probe tip. Therefore, 11.1 seconds were needed to deliver 1 J/Cm^2 to each of the two spots treated.

In order to enhance the therapeutic effects of the laser employed in this study, the rats' fur in the area of the medial gastrocnemius was removed with a commercial hair removal product " Neet ^{R} " on the first day of treatment. Following the administration of the hair removal, the rats ' hind limbs were rinsed with normal saline solution for one minute to avoid chances of this chemical product penetrating into the animals' legs. Moreover, the hair removal procedure was repeated on days four and seven of the treatment period, to ensure the penetration of the laser energy into the target tissue. After the first hair removal session, shaving was

used because it was easier since there was not a great amount of fur to be removed. The animals were positioned manually for the laser treatment. The laser was applied to the medial aspect of the gastrocnemius muscle of the right leg of all animals (traumatized area). The right foot of the rat was kept in extension by gentle traction of the distal phalanges to ease the application of the laser therapy. During the treatment time, the legs of the animals were separated in order to reduce the likelihood of having sideways effects of the laser beam on the other leg. The point technique of laser application was used. The laser beam was positioned perpendicularly to the surface area that was laser treated. The laser beam was held stationary and kept in direct contact with the treated spots. This positioning was done to avoid extra refraction and lead to better absorption (Calderhead and Ohshiro 1988). An area of 2 Cm^2 was treated. Therefore, the laser was applied to two adjacent spots for the stipulated treatment time mentioned above.

The treated areas had the same size in all animals as the same injury apparatus and technique were used throughout the experiments. All the controls were sham treated in the same manner. The laser equipment was checked for calibration at the beginning of the study (Appendix C, Figure 10).

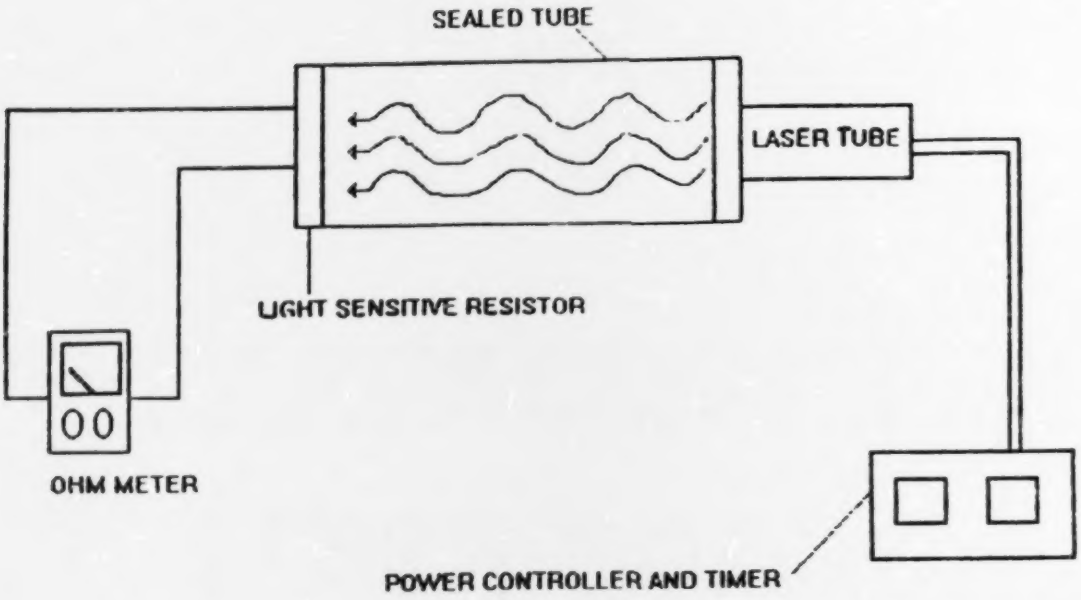

FIGURE 10: LASER CHECK CALIBRATION

STUDY DESIGN

This study was designed to evaluate the effects of Low Power Laser Therapy on skeletal muscle following acute blunt trauma, at the cellular level. In order to achieve the objectives, an animal model was employed. There were four groups of male Sprague Dawley rats with seven animals in each group (N = 28). The animals were randomly allocated to their groups. The mean initial body weight for each group was similar to ensure all the animals had about the same muscle weight in their legs.

The groups were divided as follows: Groups I and II were control groups and Groups III and IV were treatment groups. Group I was composed of control non injured animals which received no treatment; Group II was composed of injured animals which received no treatment; Group III was composed of non injured animals which were laser treated; and finally, Group IV had injured animals that were laser treated. The rationale for having two control groups was that with acute blunt trauma, there is an inflammatory reaction and associated systemic metabolic effects. By using groups that were not be treated by any therapy, it was hoped that the effects of the therapy used in the treated groups was being controlled.

The alterations in non-collagenous protein and collagenous protein content were the dependent variables of this study. The application of the LPLT and muscle injury (acute blunt trauma) were the independent variables. The

effects of LPLT and muscle injury were studied through measurements of alterations in non-collagenous protein and collagenous protein content.

The study was divided into four parts: A) Muscle samples were obtained from control groups I and II; B) Muscle samples were obtained from treatment groups III and IV; C) All muscle samples were prepared for the biochemical assays; and D) Biochemical assays were performed. All animals were sacrificed at the end of each experimental part of the study (parts A and B). Muscle tissue samples were obtained from the medial gastrocnemius muscle of all animals.

DATA COLLECTION

Muscle tissue samples from the medial gastrocnemius muscle were dissected intact from all animals at the end of each experimental period. The samples were put in separate and properly labelled vials and frozen at -70^{0}C. The samples were then freeze dried to have their water component removed. Next, they were powdered under liquid nitrogen to become homogenized. Approximately one mg of each dried sample was then weighed and prepared for the biochemical assays used in this study (Appendices A and B). The biochemical assays used were the Lowry et al. technique for the determination of non-collagenous protein content (appendix A) and the Woessner technique for the determination of collagenous protein content (appendix B). The above procedures and assays were performed

by the investigator.

ANALYSIS OF DATA

This study aimed at determining the effects of Low Power Laser Therapy on skeletal muscle following acute blunt trauma. The alterations that occurred to non-collagenous and collagenous protein contents of the muscles sampled were measured in controlled experiments. The effects of LPL therapy upon muscle non-collagenous protein and collagenous protein contents were measured in the four groups and the two-way analysis of variance was used to determine if there was any significant difference in the results. A P value < 0.05 was considered to be significant. In order to determine where any significant difference lay, Post Hoc comparisons were made (Scheffe), (see Tables 4 to 7). The data was described in terms of means, standard deviation and standard error of the mean.

SAFETY

According to Castel (1985), no documented adverse effects or any significant hazard has been reported to be associated with laser therapy. However, it was important to observe its safety requirements, which were centered on the protection of the eyes due to the high sensitivity of the retina to light energy and the focusing ability of the cornea. Therefore, throughout this study, the safety requirement inherent to this

therapeutic modality (the use of protective goggles) was followed by the investigator.

CHAPTER FOUR

RESULTS

INTRODUCTION

A controlled and reproducible experimental model of blunt trauma to skeletal muscle, developed by Stratton and associates (1984), and modified by Fisher et al. (1990), was used to study the effects of LPLT in the muscle healing process following trauma.

Following anaesthesia and trauma to the medial gastrocnemius of the right hind limb, all animals were observed to function normally inside their cages. Three days post-trauma, treatment was begun and ended on day twelve. Following the period of treatment, all animals were anaesthetized and sacrificed. The medial gastrocnemius muscle of only the right leg of each animal was then dissected intact and removed.

ANIMAL BODY WEIGHT

INITIAL WEIGHT

As shown in table 1, at the beginning of the experiment, there was no significant variation in the body weights of animals, because they were ordered through the Department of Biological Sciences to weigh between 200 and 250 grams. The raw data for initial body weight is presented in Appendix D.

Table 1: Animal Initial Body Weight in Grams.

Obs: Values are: Means, Standard Deviation (S.D) and Standard Error of the Means (S.E.M).

	CONTROL NON-INJURED	CONTROL INJURED	LASER NON-INJURED	LASER INJURED
MEANS	240.85	241.85	240.85	237.71
S.D	6.64	8.35	6.20	4.46
S.E.M	2.59	3.16	2.34	1.68

FINAL WEIGHT

Table 2 shows that at the end of the experiment prior to sacrifice, no significant difference was found among all animals ($p > .05$), which suggests that neither the anaesthetic used, nor the trauma induced, nor the treatment applied, influenced the food intake or the mobility of the animals. The raw data for the final muscle weight is presented in Appendix E.

Table 2: Animal Final Body Weight in Grams.

Obs: Values are: Means, Standard Deviation (S.D) and Standard Error of the Means (S.E.M).

	CONTROL NON-INJURED	CONTROL INJURED	LASER NON-INJURED	LASER INJURED
MEANS	324.14	327.28	341.14	351.71
S.D	10.15	12.69	32.71	14.41
S.E.M	3.84	4.80	12.39	5.46

WEIGHT OF MEDIAL GASTROCNEMIUS MUSCLE DISSECTED FROM ANIMALS

As shown in Table 3, there was no significant difference among the muscle weights sampled from the animals at the time of sacrifice. This finding confirmed the fact that the animals did not have any alteration in their food intake or mobility, which could have influenced the results of the measurements done in this study. The raw data for the medial gastrocnemius muscle weight dissected from all the animals is presented in Appendix F. Approximately one mg of dry tissue (medial gastrocnemius), see appendix G, was hydrolysed and utilized for biochemical analysis.

Table 3: Medial Gastrocnemius Muscle Weight Dissected from animals (milligrams). Obs: Values are: Means, Standard deviation (S.D) and Standard Error of the Mean (S.E.M).

	CONTROL NON-INJURED	CONTROL INJURED	LASER NON-INJURED	LASER INJURED
MEANS	514.00	559.00	497.00	577.71
S.D	74.98	61.33	59.18	60.69
S.E.M	28.40	23.23	22.41	22.98

ASSAYS

After the tissues samples were hydrolysed and prepared (redissolved and filtered), a series of standards were prepared to establish an accurate curve for each assay (see Appendices A and B). Duplicates of the final solutions had their absorbance determined based on the colour development in comparison to the established standards. The relation between absorbance and concentration was determined, and the non-collagenous protein values (Lowry et al. 1951, appendix A) and the collagenous protein values (Woessner 1961, appendix B) were determined directly from the their respective standard curves (Figures 11 and 12). Both values had a coefficient of correlation (r) of 0.99. The raw data for the non-collagenous protein (Lowry et al. 1951) and collagenous protein (Woessner 1961) assays are presented in Appendices H and I, respectively.

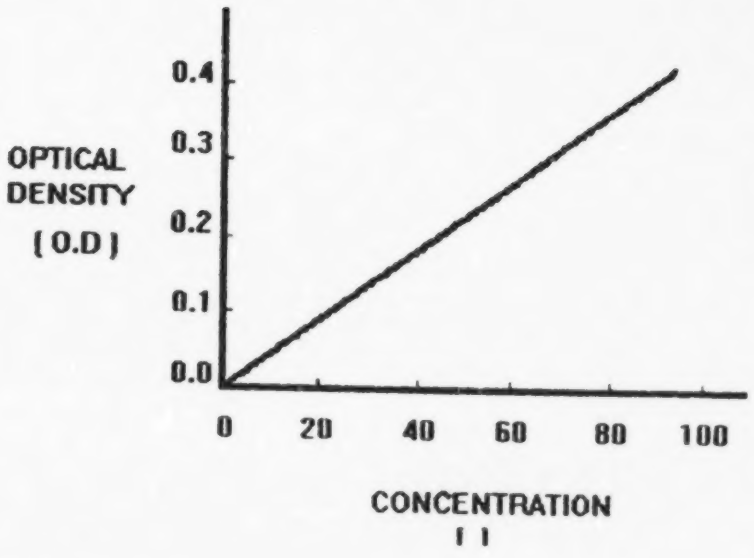

FIGURE 11: STANDARD LOWRY CURVE

(NON-COLLAGENOUS PROTEIN ASSAY)

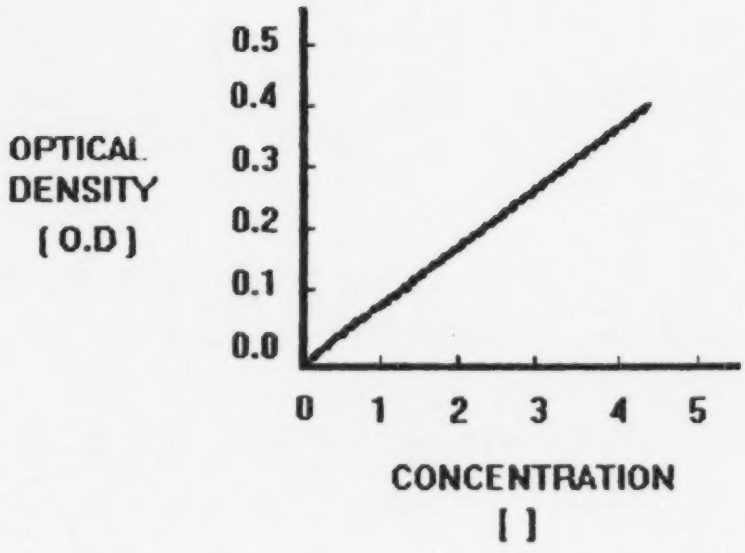

FIGURE 12: STANDARD WOESSNER CURVE

(COLLAGENOUS PROTEIN ASSAY)

NON-COLLAGENOUS PROTEIN CONTENT

In order to assess the effects of LPLT on skeletal muscle following injury, observations were made on four groups. As shown in Figure 13 and Tables 4 (Two Way Anova, $p > .05$) and 5 (Post Hoc Comparisons with Means and Standard deviations), there were no significant differences found in non-collagenous protein content among the groups. Furthermore, no significant interaction effects between injury and LPLT were demonstrated in the study (see Figure 14). There appeared to be a decrease in the non-collagenous protein content of control injured animals (Group II) as compared to control non injured animals (Group I) following the twelve day post-trauma period of this study. However, this decrease did not imply in any significant difference for non-collagenous protein between those two groups ($p > .05$), rejecting the first research hypothesis of this study.

There was no significant difference found between the levels of non-collagenous protein content of control injured animals (Group II) and lased injured animals (Group IV) as seen in Table 5. This finding indicates that LPLT did not increase the level of non-collagenous protein, rejecting the third research hypothesis of this study. Despite of no significant difference being found among the groups studied for non-collagenous protein content, the data presented in Figure 13, demonstrated that lased injured animals (Group IV) have a lower level of non-collagenous protein content as

compared to control non injured animals (Group I), control injured (Group II) and lased non injured animals (Group III).

There was no significant difference found in the levels of non-collagenous protein content between control non injured animals (Group I) and lasered non injured animals (Group III) (Table 5). Therefore the fifth research hypothesis of this study was accepted. Despite no significant difference being found among the groups studied, LPLT to non injured animals (Group III) appeared to inhibit the synthesis of non-collagenous protein, as the results in Figure 13 and Table 4 show the levels of non-collagenous protein to be lower in the laser treated groups than in the non laser treated groups.

Table 4: Two Way Anova Summary for non-collagenous protein content (Lowry et al. 1951, Appendix A).

SOURCE	M.S	F-RATIO	PROB.
INJURY	19919.274	1.436	0.256
LASER	0.000	0.000	1.000
INJURY X LASER	0.000	0.000	1.000

Table 5: Means, Standard Deviation (S.D) and Standard Error of the Means (S.E.M) for Non-Collagenous Protein Content. Post Hoc Comparisons (Scheffe) were performed, but no differences were found.

Obs: Values are in ug/mg of dry tissue.

	CONTROL NON-INJURED	CONTROL INJURED	LASER NON-INJURED	LASER INJURED
MEANS	495.85	466.85	430.14	372.14
S.D	70.53	89.63	90.87	184.97
S.E.M	26.71	33.95	34.42	70.06

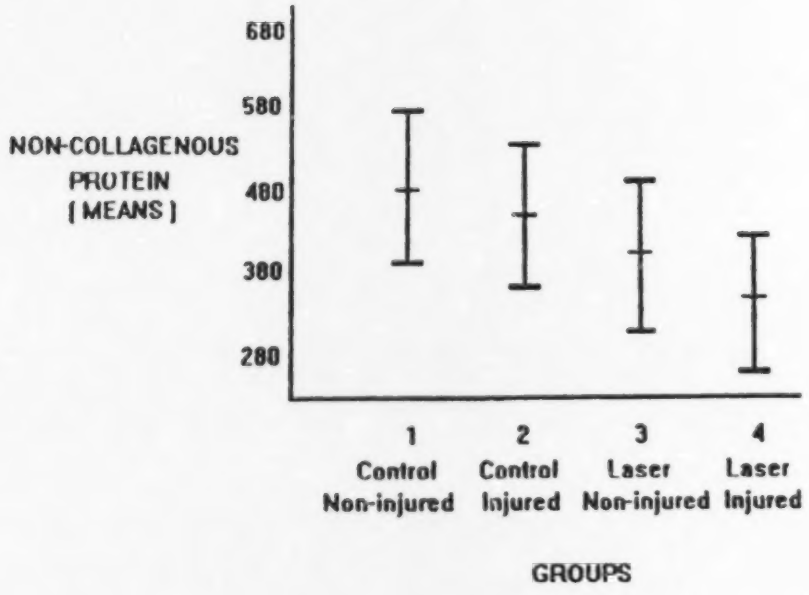

FIGURE 13: NON-COLLAGENOUS PROTEIN DATA FOR ALL GROUPS

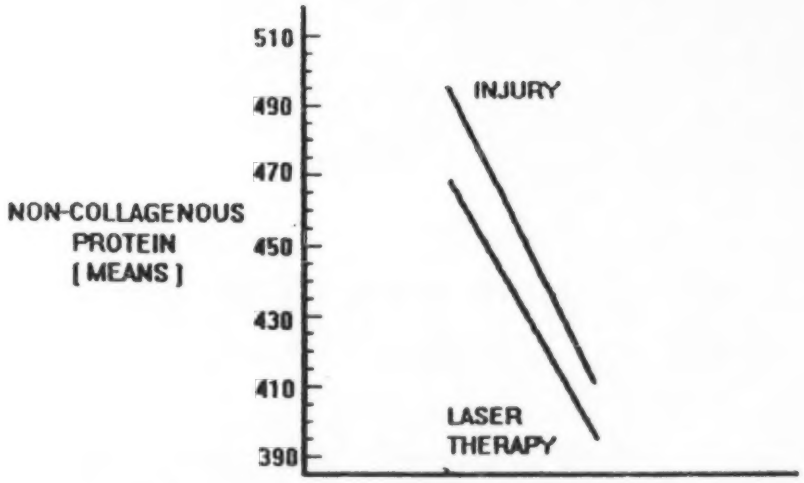

FIGURE 14: ABSENCE OF INTERACTION BETWEEN LASER THERAPY AND INJURY FOR NON-COLLAGENOUS PROTEIN DATA

COLLAGENOUS PROTEIN CONTENT

There was a significant increase in collagenous protein content of control injured animal muscle (Group II) in comparison to control non injured animal muscle (Group I) as shown in Figure 15 and Tables 6 (Two Way Anova, $P < .0005$) and 7 (Post Hoc Comparisons with Means and Standard Deviations). Therefore, the second research hypothesis of this study was accepted.

LPLT to injured animals (Group IV) apparently increased the levels of collagenous protein content in comparison to the normal healing levels of control injured animals (Group II), but there was no significant difference found between these two groups as shown in Table 7,rejecting the fourth research hypothesis. However, there was a significant difference between the group that was injured and received LPLT (group IV) and the control non injured animals (Group I) as shown in Table 7. Moreover, LPLT to injured animals (Group IV) apparently increased their level of collagenous protein content in comparison to LPLT to non injured animals (Group III), but it was not significant (Table 7).

In addition, LPLT to non injured animals (Group III) significantly increased ($p < .005$) the level of collagenous protein content in comparison to control non injured animals (Group I) (Table 7) rejecting the sixth research hypothesis of this study. Figure 16 demonstrates that there were no interaction effects between injury and LPLT used in the study.

Table 6: Two Way Anova Summary for Collagenous Protein Content (Woessner 1961, Appendix B).

SOURCE	M.S	F-RATIO	PROB.
INJURY	27.058	18.836	0.000
LASER	0.000	0.000	1.000
INJURY X LASER	-3.55	0.000	1.000

Table 7: Means, Standard Deviation (S.D) and Standard Error of the Means (S.E.M) for Collagenous Protein Content and Results from Post Hoc Comparisons (Scheffe). Post Comparisons were performed and the group marked by a * was significantly different from the groups marked by * * .

Obs: Values are in ug/mg of dry tissue.

	I CONTROL NON-INJURED	II CONTROL INJURED	III LASER NON-INJURED	IV LASER INJURED
MEANS	2.78 *	5.04 * *	4.28 * *	5.29 * *
S.D	0.35	1.14	0.40	0.56
S.E.M	0.13	0.43	0.15	0.21

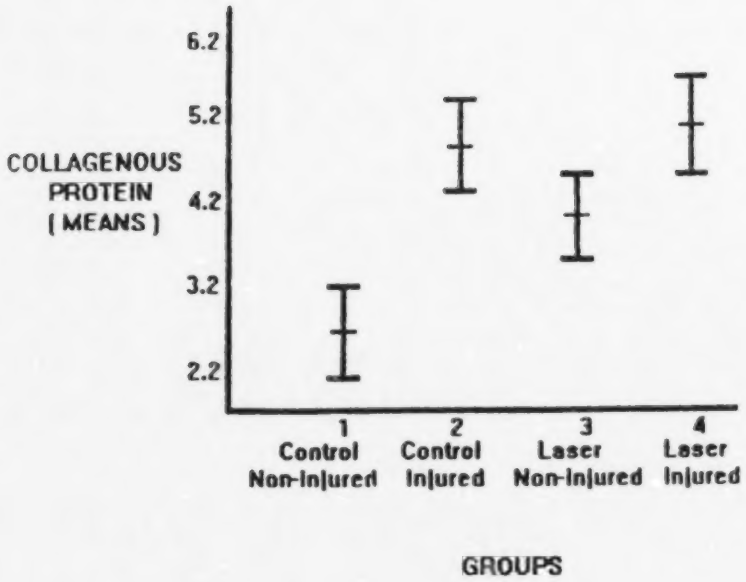

FIGURE 15: COLLAGENOUS PROTEIN DATA FOR ALL GROUPS

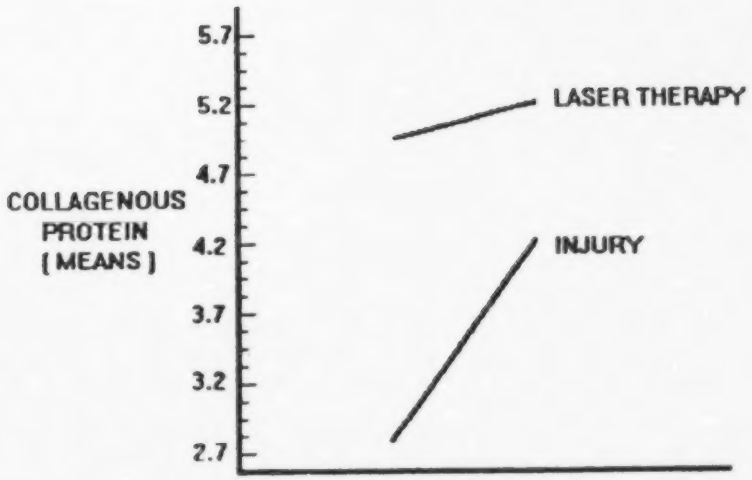

FIGURE 16: ABSENCE OF INTERACTION BETWEEN LASER THERAPY AND INJURY FOR COLLAGENOUS PROTEIN DATA

CHAPTER FIVE

DISCUSSION

LOW POWER LASER THERAPY FOR MUSCLE TRAUMA

As mentioned in the review of the literature, there are a variety of laser devices and parameters used in clinical and scientific investigations (Enwemeka 1988, Woolley-Hart 1988, Basford 1989). Therefore, a comparison of such results is extremely difficult and may not be complete. Standardization of parameters, devices and methods used to assess the effectiveness of LPLT is of importance if the results of studies are to be meaningful and applicable (Basford 1989). In this study, a reproducible model of acute blunt trauma (Stratton et al. 1984, Fisher et al. 1990), established assays for determining non-collagenous and collagenous protein content, Lowry et al. 1951 and Woessner 1961 respectively, and a versatile LPLT device (Lazer Train System IV - Sterne) were utilized. The combination of these elements may provide for valid and reliable results as well as for the standardization of parameters of the dosage of LPLT used to treat the muscle trauma addressed by this study.

The dosage chosen for this study was based on a review of the literature, which still has not been standardized. Following injury, there is an inflammatory response which lasts from three to five days (Gould 1990). At this stage, LPLT has been reported to increase macrophage activity

(Enwemeka 1988) which clears the injury site of cellular debris and necrotic tissue that may be present.

In this study, LPLT was applied from day 3 post-injury to day 12, that is, during the fibroblastic phase of post-injury repair, which lasts for weeks (Gould 1990, Reed and Zarro 1990). According to the literature (Mester et al. 1985, Lyons et al. 1987), LPLT applied during this period provides an increase in the collagenous protein content production, which leads to the strengthening of the wound. Because of these assumptions, LPLT was expected to lead to an increase of both the levels of non-collagenous and collagenous protein content.

Based on the findings of Mester et al. (1985), and on the observations made by other authors (Castel 1985, Calderhead and Ohshiro 1988, Enwemeka 1988, Basford 1989), when LPLT was applied at a dosage of 0 to 4 j/cm^2, it had stimulative effects, but when it exceeded 4 j/cm^2, it had inhibiting effects. It is not clear, however, how the energy delivered by the LPLT might have cumulative and/or deleterious effects.

As indicated in Chapter II, in order for regeneration to be effective, it is important that the muscle cell basal lamina remain intact after injury, so as to prevent the infiltration of fibroblasts, which compete with myoblasts and satellite cells and result in the formation of scar tissue. In this study, the increase in collagenous protein in the injured animals of group IV following LPLT could not be related to this factor because collagenous protein also increased in the

non-injured animals of group III.

It was hoped that LPLT would not lead to fibroblast proliferation to the point of replacing the damaged structures with fibrous tissue which is non-functional, but would lead to an increase in the activity or proliferation of myoblasts leading to the reconstitution of muscle protein.

POSSIBLE MECHANISMS OF ACTION OF LPLT

In the catabolic response of muscles following trauma, proteins are broken down (Carlson and Faulkner 1983, Tischler et al. 1983, Rennie 1985, Fisher et al. 1990), followed by an anabolic response with the formation of new muscle fibers and/or scar tissue depending on whether the sarcolemma remains intact (Engel and Banker 1986). Therefore, the outcome of the anabolic response is dependent upon the integrity of the sarcolemma. When the sarcolemma is intact, satellite proliferation and myonuclei of muscle fibers form myoblasts which fuse into myotubes, forming myofilaments, which then form new muscle fibers. Non-collagenous protein content then gradually returns to its normal level. During the anabolic response however, even with the sarcolemma intact, some collagenous proteins have to be present because they form a sheath around fusing myotubes during myoblast formation. These proteins are produced by fibroblasts or by other cells resembling fibroblasts in the early stages (24 to 48 hours) of wound healing, but with no definite morphology of fibroblasts

(Gay et al. 1978). In addition, collagenous proteins are produced at a faster rate than non-collagenous proteins, which may be a reason for the formation of scar tissue.

According to the present data, LPLT may have stimulated collagen production. However, it was not clear why LPLT did not stimulate non-collagenous protein content in a similar manner, because the energy delivered by the LPLT theoretically passed through different layers of muscle tissue stimulating the tissue as a whole. Moreover, the assays utilized were indicative of the whole collagenous and non-collagenous protein content of the muscle.

In contrast, if the sarcolemma was torn following injury, there would be infiltration of muscle with fibroblasts with proliferation of collagenous protein and formation of scar tissue. According to the findings reported in this study, LPLT may have stimulated the cells responsible for collagenous protein formation. These cells were already proliferating and were produced at a faster rate than the others responsible for muscle fiber formation (Engel and Banker 1986). Therefore, the results would be higher for collagenous protein content and possibly not be completely beneficial since it would probably lead to the formation of scarred, non-functional tissue. In this study, however the integrity of the sarcolemma was not determined. Therefore, additional studies appear warranted.

During wound healing, cellular events interact and lead

to restoration of the integrity of the injured tissue (Gould 1990). Macrophages and T-lymphocytes and their respective growth factors have been reported to influence healing (Shurman et al. 1989). Interlukin-1 (IL1) and tumor necrosis factor alpha are the major growth factors (monokines) released by macrophages and these factors have been associated with the earliest phase of wound healing, which is inflammation (Barbul 1989). Moreover, in In Vitro experiments, monokines have been reported to influence the proliferation of fibroblasts (Schimidt et al. 1982) and to stimulate the synthesis of collagen (Kahari et al. 1987).

Interferon-y (IFN-Y) and transforming growth factor-beta (TGF-B) are two well known growth factors (Lymphokines) released by lymphocytes. Besides being a fibroblast chemotatic molecule, TGF-B may induce chemotaxis of monocytes and secretion of fibroblasts growth factors such as IL-1 (Whal et al. 1987). Therefore, lymphocytes may lead to an increase in the synthesis of collagenous protein. However, IFN-Y has also been related to the inhibition of collagen synthesis (Barbul 1989). Thus, cells such as macrophages and lymphocytes which infiltrate the injured tissue before the fibroblastic phase takes place, have an important role in the development of the fibroblast population and its synthesis of collagenous protein.

In this study, following trauma, besides the possibility of having the sarcolemma torn with subsequent infiltration and

proliferation of fibroblasts, it might be that the macrophages and lymphocytes growth factors might have been activated, which led fibroblasts to synthesize more collagenous protein as outlined in the data.

NON-COLLAGENOUS PROTEIN CONTENT

It is known that muscle fibers are mainly constituted of proteins, which undergo processes of continuous degradation and replacement by synthesising new proteins (Rodemann and Goldberg 1982). Therefore, in order to determine the level of protein in tissues, the rates of protein degradation and synthesis are used and their balance determines whether a muscle is undergoing atrophy or growth (Tischler and Fagan 1983).

Skeletal muscle tissue contributes to a great extent to amino acid and protein metabolism (Rennie 1985). However, the exact contribution of muscle to protein metabolism is not known and the changes in muscle protein during and following injury are not known in detail (Rennie 1985). According to Rennie (1985), following moderate trauma, the rate of protein breakdown is greater than the rate of protein synthesis, and following severe trauma, there are rapid increases in the rates of protein synthesis but these increases are exceeded by the rates of protein breakdown. Rennie (1985), however, did not define moderate or severe trauma.

Measurements of muscle amino acid balance give

information about individual processes of synthesis and breakdown, but only of their difference (Rennie 1985). However, in the present study, only observations of the level of non-collagenous protein content were made and the data demonstrated that it was apparently lower than the controls following trauma.

Prostaglandins have been reported to be involved in the regulation of the synthesis and degradation of skeletal muscle protein (Rodemann and Goldberg 1982, Barnett and Ellis 1987). Prostaglandins (PGs) may be produced by muscle cells when these cells have their cell membrane integrity altered by injury. Thus PGs are involved in the process of inflammation. Fisher et al. (1991), demonstrated that following muscle injury there was a systemic inflammatory response, which was associated with increased synthesis of PGE_2. According to Rodemann and Goldeberg (1982), enhanced PGE2 and PGF2 alpha in isolated rat muscles, led to stimulation of protein degradation and synthesis, respectively. However, Barnett and Ellis (1987), did not find PGE_2 to influence protein degradation in isolated muscles. Therefore, there is still need for future studies to clarify the effects of PGs upon muscle protein metabolism following injury.

In the catabolic response, following muscle trauma, the levels of non-collagenous protein content are reduced (Tishler and Fagan 1983, Rennie 1985, Fisher et al. 1991). In this study, the effects of acute blunt trauma and the effects of

the application of a therapeutic modality (LPLT, daily x 10 at $1j/cm^2$) were determined from the results found when the animals were sacrificed at the end of the experiment.

Fisher et al. (1990) demonstrated that non-collagenous protein content was reduced by 16% within 24 hours post-trauma, and that this reduction was further augmented for three days following trauma. The data from the present study did not show any significant difference between the levels of non-collagenous protein content 12 days post-trauma (Figure 13). However, non-collagenous protein content of control injured animals (Group II) appeared to be lower than that of control non injured animals (Group I) following a period of 12 days post-injury. It is known that following a catabolic response due to trauma, muscles undergo an anabolic response and according to Fisher et al. (1990), non-collagenous protein content from injured animals will only reach the same level of their controls at approximately 21 days post-injury.

In the present study, even though the non-collagenous protein content results for control non injured and control injured animals was not found to be different, they agreed with the first research hypothesis of this study which stated that injury decreased the level of non-collagenous protein content in skeletal muscle. The possible explanation for the above mentioned data is that the level of non-collagenous protein content of control injured animals was still rising at day twelve, to possibly reach the level of its control non

injured as previously shown by Fisher et al. (1990).

The level of non-collagenous protein content of control injured animals (Group II) was higher than that of the lased injured animals (Group IV), but it was not significantly different ($p > .05$), rejecting the third research hypothesis and demonstrating that LPLT was not of any benefit to non-collagenous protein content (Figure 13 and Table 5) in the circumstances of this study. It may be that the LPLT protocol used led to an inhibition to the synthesis of non-collagenous protein, which means that the dosage chosen might not have been the most appropriate. However, the mechanisms of this possible inhibition were not addressed and cannot be explained by this study. It appears that the inhibition caused by the LPLT was also in agreement with the data for lased non injured animals (Group III), which had their levels of non-collagenous protein lower than their control non injured animals (Group I) as shown in Figure 13 and Table 5. However, there was no significant difference between these groups and the fifth research hypothesis was accepted. A more reasonable explanation for the lower level found in the injured animals treated with LPLT may have been the competition between collagenous and non-collagenous protein production that takes place post-injury (Allbrook 1973). Moreover, the data for the non-collagenous protein content agreed with the data for the collagenous protein content in which the levels increased significantly ($P < .005$) in the injured and treated groups in

comparison to the control non injured group.

COLLAGENOUS PROTEIN CONTENT

According to Lehto et al. (1986), collagen production starts two days post trauma, is most intensive between days 5 and 21 and decreases thereafter. The present study was limited to 12 days post-trauma. The data from Figure 15 and Table 7 confirmed the second research hypothesis that injury caused an increase in the level of collagenous protein content in skeletal muscle of rats when control injured animals (Group II) were compared to control non injured animals (Group I) ($p < .005$). This data is in agreement with ultrastructural (Electronmicroscopic) findings of proliferation of macrophages and fibroblasts at the site of injury (Lehto et al. 1986, Fisher et al. 1990).

When looking at the difference between control injured animals (Group II) and lased injured animals (group IV), no significant difference was found for collagenous protein, despite the trend of LPLT to increase the levels of this type of protein. Therefore, the fourth research hypothesis of this study that LPLT to injured animal muscle increases the level of collagenous protein content as compared to normal healing levels was rejected. This tendency of LPLT to increase collagenous protein is not beneficial at this point in time, when an injured muscle is undergoing its anabolic phase for non-collagenous protein content, to reassume its normal

characteristics. On the contrary, a greater increase in non-collagenous than in collagenous protein content at twelve days post-trauma was expected. However, the literature has demonstrated LPLT to increase the synthesis of collagen (Mester et al. 1985, Lyons et al. 1987). In Mester's et al. work (1975), despite of their positive results (chapter II) in treating muscle injuries, adverse effects caused by LPLT were reported and related to the formation of excess of collagen.

Even though the present study and that of Mester and associates (1975) evaluated the effects of LPLT upon muscle injury, they cannot be compared, because that of Mester et al. (1975) utilized a different injury model (incisions) and a laser with a lower depth of penetration. Although the work by Mester and associates (1975), demonstrated that LPLT may be of benefit in the healing process following muscle injury, the data from this study did not confirm this fact. Considering the time frame for treatment used in this study, the LPLT protocol used may not be of benefit, because like the findings of Mester et al. (1975), it may adversely affect the quality of the healing process in the injured muscle. Therefore, the protocol used in the present study would probably be of benefit at a later stage, after the first three weeks post-trauma in which other forms of treatment, such as ice and exercise could be used.

In the present results, however, it appeared that LPLT to injured animals (Group IV) yielded higher levels of

collagenous protein as compared to the other groups. A possible explanation for the increase in collagenous protein in the lasered injured animals (Group IV) may be the fact that these injured animals underwent a catabolic response due to the injury, followed by an anabolic response which activated the production of collagenous protein, whereas animals in group III which also received laser, had not suffered any injury. This data agreed with the data from control injured animals (Group II), but showed that LPLT to non injured animals (Group III) still had an effect upon collagenous protein content when compared with control non injured animals (Group I) ($P < .005$), rejecting the sixth research hypothesis of this study that LPLT to uninjured muscle has no effect upon collagenous protein content. Therefore, it appears that the dosage of LPLT has to be carefully designed to meet the needs of various clinical conditions and the period during which this modality is applied.

CHAPTER SIX

CONCLUSIONS

INTRODUCTION

Muscle injuries are frequent among sports participants (Smodalaka 1979) and the extent of the damage caused is difficult to be measured scientifically. As LPLT is being employed as a therapeutic modality by therapists to treat sports injuries (Gordon 1990), this study was aimed at determining its effectiveness in the treatment of single acute blunt trauma.

Twenty eight male Sprague Dawley laboratory rats were assigned to four groups, which consisted of control non-injured, control injured, laser non injured and laser injured animals with seven rats in each group. LPLT was applied at a dosage of 1 j/cm^2 to two adjacent spots in the medial gastrocnemius, daily for ten days. Measurements of the changes in the levels of A) non-collagenous protein and B) collagenous protein were determined twelve days post trauma.

The present results demonstrated a general, but not significant, decrease in non-collagenous protein and an overall and significant increase in collagenous protein content ($P < 0.005$).

SUMMARY OF THE RESEARCH HYPOTHESES STUDIED

In summary, based on the results of this study:

1 The first research hypothesis was rejected: although the results did not show any significant difference, rejecting this hypothesis, there was a trend for injury to cause a decrease in the level of non-collagenous protein of skeletal muscle.

2 The second research hypothesis was accepted: the hypothesis that injury causes an increase in the level of collagenous protein in skeletal muscle was accepted.

3 The third research hypothesis was rejected: LPLT to injured animal muscle did not increase the level of non-collagenous protein content as compared to normal healing.

4 The fourth research hypothesis was rejected: LPLT to injured animal muscle did not significantly increase the level of collagenous protein content as compared to normal healing.

5 The fifth research hypothesis was accepted: LPLT to non injured animal muscle had no effect upon the level of non-collagenous protein content.

6 The sixth research hypothesis was rejected: LPLT to non injured animal muscle increased the level of collagenous protein content.

CLINICAL RELEVANCE OF THE STUDY

In the present study, in view of the non-significant differences found for the non-collagenous protein data, it is

not possible to completely ascertain whether LPLT may or may not be deleterious for the restitution of non-collagenous protein in injured muscle.

The data for collagenous and non-collagenous protein showed an opposite trend. Therefore, one is led to believe that LPLT following trauma under the conditions of this study, is not beneficial because it may lead to the formation of fibrotic, non-functional scar tissue in the skeletal muscle. However, it must be considered that only one treatment protocol was used and only one time frame relative to the injury was studied. Moreover, different parameters may be used to evaluate the efficiency of a therapeutic modality. Therefore, further studies are required to demonstrate the influence of LPLT upon non-collagenous and collagenous protein content and other functional characteristics of skeletal muscle tissue, following acute blunt trauma.

RECOMMENDATIONS

It is suggested that future studies look at different protocols and measure the effects of LPLT at different points in time to ascertain how this therapeutic modality may affect the healing process of an acute blunt trauma to skeletal muscle.

Experimental clinical use of LPLT protocols at different stages of post-traumatic injuries may be justified by future studies.

CONCLUSIONS

The following conclusions may be stated within the limitations of this study:

1 The present data for animal muscle weight confirmed that the model of acute blunt trauma used does not cause any alteration in either food intake or mobility of the animals which could influence the outcome measures of this study.

2 The present data for non-collagenous and collagenous protein content indicate that the LPLT protocol used should not be used to treat acute blunt trauma to skeletal muscle since, it may lead to the formation of fibrotic, non functional tissue in the muscle.

3 The model used may serve as a basis for further studies of muscle protein metabolism in trauma and of the effects of different therapeutic modalities used by physical therapists during the healing process.

4 Further studies are warranted to evaluate the effects of LPLT upon acute blunt trauma to skeletal muscle tissue and other tissues under different clinical conditions.

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APPENDIX A

METHOD OF LOWRY

In 1951, Lowry and associates developed a simple and sensitive method for the measurement of protein in solution or suspension. To ensure solubilization of proteins, the sample is made alkaline with KOH. In an alkaline environment, Cu^{2+} ions bind to the carbonil (-C=O) and imine (=N-H) groups of the peptide bonds. This complex acts as a reducing agent to change the structure of the Folin reagent such that it changes colour from light yellow to a deep blue. The extent of this colour development can be quantified by measuring the absorbance of light at a wavelength of 750 nm. By constructing a standard curve with the protein bovine serum albumin (BSA), protein concentrations in sample solutions can be estimated based on colour development in comparison to the standards.

REAGENTS

1 5% cupric sulfate ($CuSO_4$ $5H_2O$).

2 10% sodium potassium tartrate ($NaKC_4$ H4 O_6).

3 10% sodium carbonate (Na_2 CO_3),pH to 12.5 with NaOH.

4 LOWRY C solution:

.1 ml 10 % Sodium Tartrate (fresh) 50 mg/.5ml d H2O

.1 ml 5% Cupric Sulfate (stock)

10 ml Sodium carbonate in 2% NaOH (stock)

5 Folin and coicateau phenol reagent, diluted 1 : 1 with H_2O.

6 Protein standard stock solution: 1 ug/ml BSA in H_2O.

7 Pipets, test tubes and racks, shaking water bath, vortex mixer, spectrophotometer, beakers, graduated cylinder, cuvettes and marking pen.

STANDARD CURVE

STOCK (ml)	WATER (ml)	CONCENTRATION (ug/ml)
0	0.5 ml	0
0.1	0.4 ml	1
0.2	0.3 ml	2
0.3	0.2 ml	3
0.4	0.1 ml	4
0.5	0 ml	5

PROTOCOL

1 SOLUTIONS

A - Lowry C solution

B - 1 ml Folin and 1 ml d H_2O

2 ASSAY

a) Take sample volumes up to .5ml

b) Add .1 ml of solution A while vortexing

c) After exactly 10 minutes, Add .05 ml of solution B while vortexing

d) Let stand at room temperature for 30 minutes.

3 SPECTROPHOTOMETRIC ANALYSIS

a) set spectrophotometer wavelength to 750 nm.

b) read and record optimal density of each sample.

c) plot standard curve (x = protein concentration, y = optical density) and estimate unknown protein concentrations.

Tissues samples were hydrolysed in 6 MHCL at 105 degrees celsius for 24 hours. Approximately 1 mg of tissue was weighed and 1 ml of MHCL was added. The acid was removed after hydrolysis in vacuum. The samples were redissolved in DH_2O (1 ml) and filtered.

After the samples were prepared, a series of standards were prepared to establish an accurate curve. The final solutions had their absorbance determined based on the colour development in comparison to the standards. The relation between absorbance and concentration was found and the non-collagenous protein values were determined directly from the standard curve.

APPENDIX B

METHOD OF WOESSNER

This method is a determination of Hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Hydroxyproline is used as an indicator of the presence and of the metabolism of collagen due to its restricted distribution in animal organisms. In 1961, Woessner developed a method for the determination of hydroxyproline in biological materials in which there is a small proportion of hydroxyproline to other amino acids. The Woessner method is based on modifications performed by other investigators with the intent of eliminating variations that may be present. In order to avoid any variations the starting material was purified before any analysis was performed.

REAGENTS

1 Citrate Buffer

50 g (citric acid. H_2O) + 120 g Sodium Acetate. 3 H_2O + 34 g NaOH + 12 ml of glacial acetic acid and dilute to 1 liter with DH_2O. Adjust pH to 6.00 and refrigerate. (shelf life = 1 month).

2 Chloramine T (fresh daily)

0.4 g Chloramine T (kodak) + 2 ml of DH_2O + 3 ml of methyl cellosolve + 5ml of citrate buffer.

3 Perchloric Acid (19% v/v)
Stock Perchloric Acid is 70%

4 P - Dimethylamino Benzaldehyde (P - DMABA)
Fresh daily (20 g P - pond to the laser device to be used.

PROTOCOL

1 Prepare a stock solution of 30 ug/ml of L - Hydroxyproline

2 Pipet 0.5 ml of your standard containing 1 - 5 ug of L - Hydroxyproline.

3 At timed intervals, add 0.5 ml of chloramine T and vortex.
Allow oxidation to take place for 20 minutes at room temperature.

4 At timed intervals, add 0.5 ml of Perchlorate solution at precisely 20 minutes. Stop oxidation and let samples stand at room temperature no longer than 5 minutes.

5 Add 0.5 ml of P - DMABA and mix well. Incubate in dark at 60 degrees celsius for maximum chromophore development (20 minutes).

6 Allow samples to equilibrate to room temperature and mix before reading at 557 nm.

Tissues samples were hydrolysed in 6 MHCL at 105 degrees celsius for 24 hours. 1 mg of tissue was weighed and 1 ml of

MHCL was added. The acid was removed after the hydrolysis in vacuum. The samples were redissolved in DH_2O (1 ml).

After the samples were ready, a series of standards were prepared to establish an accurate curve. The final solutions had their absorbance determined based on the colour development in comparison to the standards. The relation between absorbance and concentration was found and the hydroxyproline values were determined directly from the standard curve.

APPENDIX C

PROTOCOL FOR LASER CALIBRATION

The lazer train unit was checked for calibration. Its specifications were provided by the manufactures manual. A steel sealed tube opened at both ends for the connection of the laser tube (delivery guide) and of a light sensitive resistor respectively, were used. An Ohm Meter (Beckman) was connected to the light sensitive resistor which was connected to the sealed tube above mentioned. The Ohm Meter was used to check for the presence and incidence of the laser beam of the laser device which was used in this study.

1 The equipment was set as shown in Figure 10

2 The power controller and the timer seen in figure 1 correspond to the laser device used in this study

3 As the power and the time are changed, the light sensitive resistor sensed different resistances, which were read in the Ohm Meter which was connected to it

4 The resistances were then noted and the measures were repeated to control for any possible interference and check for consistence of the method and the device

APPENDIX D

INITIAL ANIMAL BODY WEIGHT IN GRAMS

RAT #	CONTROL NON-INJURED	CONTROL INJURED	LASER NON-INJURED	LASER INJURED
1	230	240	235	238
2	240	235	239	241
3	249	238	245	230
4	244	241	231	244
5	248	260	246	235
6	240	237	242	237
7	235	242	248	239

APPENDIX E

FINAL ANIMAL BODY WEIGHT IN GRAMS

RAT #	CONTROL NON-INJURED	CONTROL INJURED	LASER NON-INJURED	LASER INJURED
1	317	340	340	338
2	317	312	382	371
3	320	331	355	362
4	339	330	370	347
5	312	345	286	343
6	334	315	340	366
7	330	318	315	335

APPENDIX F

MEDIAL GASTROCNEMIUS MUSCLE WEIGHT IN Mg

RAT #	CONTROL NON-INJURED	CONTROL INJURED	LASER NON-INJURED	LASER INJURED
1	506	608	559	550
2	448	605	585	500
3	445	585	500	640
4	460	500	450	637
5	630	495	415	510
6	505	630	495	572
7	604	490	475	635

APPENDIX G

MEDIAL GASTROCNEMIUS WEIGHT IN Mg USED FOR ASSAYS

RAT #	CONTROL NON-INJURED	CONTROL INJURED	LASER NON-INJURED	LASER INJURED
1	0.98	1.04	1.07	1.02
2	0.98	0.99	1.00	0.99
3	1.06	1.01	0.96	1.02
4	1.04	0.96	0.99	1.06
5	0.99	1.07	1.05	0.94
6	1.00	1.02	1.00	1.02
7	1.04	0.99	1.00	1.02

APPENDIX H

RAW DATA FOR NON-COLLAGENOUS PROTEIN (LOWRY)

NUMBER & GROUP	O.D 1	O.D 2	MEAN O.D 1-2	[ ]	WEIGHT	FINAL mg/Mg
1 C	.223	.230	.226	515.82	0.98	526.4
2 C	.227	.224	.220	500.92	0.98	511.4
3 C	.178	.177	.178	396.66	1.06	374.2
4 C	.231	.224	.227	518.30	1.04	498.4
5 C	.226	.226	.226	515.82	0.99	521.0
6 C	.252	.265	.259	597.74	1.00	597.7
7 C	.198	.209	.204	461.20	1.04	443.4
8 T	.137	.136	.137	294.88	1.04	283.5
9 T	.225	.231	.228	520.78	0.99	526.0
10 T	.204	.207	.206	466.17	1.01	461.5
11 T	.225	.247	.236	540.64	0.96	563.1
12 T	.240	.235	.238	545.60	1.07	509.9
13 T	.210	.200	.205	463.68	1.02	454.5
14 T	.203	.207	.205	463.68	0.99	468.3
15 LC	.169	.180	.175	389.21	1.07	363.7
16 LC	.224	.212	.218	495.96	1.00	495.9

APPENDIX H - CONTINUED

17 LC	.174	.185	.180	401.62	0.96	418.3
18 LC	.130	.121	.126	267.57	0.99	270.2
19 LC	.217	.205	.211	478.58	1.05	455.7
20 LC	.200	.208	.204	461.21	1.00	461.2
21 LC	.234	.241	.238	545.60	1.00	545.6
22 LT	.093	.090	.092	183.17	1.02	179.5
23 LT	.160	.153	.157	344.53	0.99	348.0
24 LT	.119	.107	.113	235.30	1.02	230.6
25 LT	.236	.234	.235	538.16	1.06	507.6
26 LT	.257	.290	.274	634.97	0.98	647.9
27 LT	.094	.094	.094	188.13	1.02	184.4
28 LT	.232	.219	.226	515.82	1.02	505.7

C denotes control animals,

T denotes trauma animals,

LC denotes laser control animals,

LT denotes laser trauma animals,

O.D stands for optical density read at the spectrophotometer,

[ ] means concentration found based on the standard curve,

WEIGHT means muscle sample weight used for the assay,

FINAL means final concentration found in ug/mg of dry tissue.

APPENDIX I

RAW DATA FOR COLLAGENOUS PROTEIN (WOESSNER)

NUMBER & GROUP	O.D 1	O.D 2	MEAN O.D1-2	[ ]	WEIGHT	FINAL mg/Mg
1 C	.047	.050	.049	3.33	0.98	3.40
2 C	.044	.042	.043	2.94	0.98	3.00
3 C	.047	.045	.046	3.12	1.06	2.95
4 C	.035	.039	.037	2.65	1.04	2.55
5 C	.035	.037	.036	2.61	0.99	2.64
6 C	.039	.029	.034	2.44	1.00	2.44
7 C	.040	.032	.036	2.58	1.04	2.48
8 T	.060	.056	.058	4.11	1.04	3.96
9 T	.060	.068	.064	4.55	0.99	4.60
10 T	.061	.067	.064	4.55	1.01	4.51
11 T	.061	.057	.059	4.18	0.96	4.35
12 T	.116	.120	.118	7.25	1.07	6.78
13 T	.107	.111	.109	6.74	1.02	6.61
14 T	.064	.061	.063	4.47	0.99	4.52
15 LC	.054	.062	.058	4.08	1.07	3.82
16 LC	.055	.055	.055	3.88	1.00	3.88

APPENDIX I - CONTINUED

17 LC	.063	.059	.061	4.30	0.96	4.48
18 LC	.058	.053	.056	3.98	0.99	4.02
19 LC	.069	.076	.073	5.17	1.05	4.92
20 LC	.061	.059	.060	4.28	1.00	4.28
21 LC	.068	.062	.065	4.61	1.00	4.61
22 LT	.070	.076	.073	5.17	1.02	5.07
23 LT	.103	.104	.103	6.38	0.99	6.44
24 LT	.077	.080	.079	5.58	1.02	5.47
25 LT	.079	.082	.081	5.75	1.06	5.42
26 LT	.071	.063	.067	4.72	0.98	4.82
27 LT	.070	.070	.070	4.93	1.02	4.83
28 LT	.075	.072	.072	5.13	1.02	5.03

C denotes control animals,

T denotes trauma animals,

LC denotes laser control animals,

LT denotes laser trauma animals,

O.D stands for optical density read at the spectrophotometer,

[ ] means concentration found based on the standard curve,

WEIGHT means muscle sample weight used for the assay,

FINAL means final concentration found in ug/mg of dry tissue.